SELF SELECTION OF DIET

VI. THE NATURE OF APPETITES FOR B VITAMINS [1]

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ONE FIGURE

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It was shown in a previous paper that appetites for diets containing thiamine, riboflavin, or pyridoxine were developed by rats previously fed diets deficient, respectively, in these vitamins, while normal animals showed no such preferences (Scott and Quint, '46b). It was concluded that appetites for these vitamins were related to nutritional need for them, and thus that they were either learned appetites or true hungers. As one of the principal differences between learned appetites and true hungers it was postulated that learned appetites for nutritional essentials require the animal to have previous experience of the effects of the beneficial diet, in order to learn to associate such effects with the diet, while true hungers are always coexistent with nutritional need and should require no learning process. Harris et al. ('33) concluded that the appetite for thiamine was learned as a result of beneficial experience. This appeared probable since rats which had previously been on a thiamine-deficient diet and had then formed the habit of eating a food of a particular flavor containing thiamine, showed no appetite for thiamine in food of a different flavor.

The present report outlines a method of determining whether an appetite such as that observed in these researches

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is learned, i.e., is a behavior conditioned by previous experiences, or represents a true hunger. The method was applied to the appetites for thiamine, riboflavin, and pyridoxine, with the result of showing their essential similarity as learned appetites.

EXPERIMENTAL

To decide whether the vitamin appetites were learned or whether they were true hungers, advantage was taken of the fact that establishment of a learned appetite for a vitamin may conceivably result in an association of beneficial effect of the vitamin with some recognizable physical characteristic of the diet, while a true vitamin hunger should be an appetite for the vitamin itself, regardless of physical characteristics of the food.

The general plan of the experiments was to allow deficient animals their choice of 2 diets of distinctly different flavors, A (anise) and O (unflavored). The A flavored diet contained the vitamin, the O flavored diet contained none. After a few days the choices were changed so that the A flavored diet contained no vitamin but the O flavored diet did. If the animals continued to eat the A flavored diet when it now no longer contained vitamin, then the appetite was learned as defined by Harris et al. ('33), but if they now ate the O diet, the result was evidence of a true hunger.

In practice, this simple scheme was considerably modified to eliminate several possible variables: (1) While we have as yet found no sex differences in self selection behavior, these possible differences were eliminated by dividing the animals according to sex as evenly as possible. (2) Although previous experiments (Scott and Quint, '46a) had indicated that there was no appetite in rats for anise flavor, this possibility was eliminated by giving the vitamin-containing food to half the animals as a choice accompanied by flavor and to the other half unaccompanied by flavor at the start of the experimental period. (3) The same experiments had indicated that there was no effect of previous diet on appetite for anise. This pos-

sibility was eliminated, however, by feeding half the deficient animals an anise-flavored, vitamin-free diet and the other half an unflavored vitamin-free diet in the control period and by feeding half the normal animals a flavored vitamin-containing diet and the other half a similar diet but unflavored in the control period. (4) The flavor was changed with respect to the vitamin not once but 3 times. This gave additional data, particularly with respect to the extent and persistence of the learned eating process. (5) A group of normal animals was included in the experiment to serve as controls, and thus demonstrate clearly the effect of deficiency on the results. (6) The data obtained with all deficient animals were averaged as were the data with all normal animals. This resulted in the cancellation of the effects, if any, of sex, flavor, and previous diet as such, and allowed the uncomplicated estimation of the effect of deficiency in influencing association of some property of the vitamin with flavor of the food.

In each of 3 experiments, with thiamine, riboflavin, and pyridoxine, respectively, 10 male and 10 female weanling rats were therefore divided into 4 groups. Each group was fed during a 3-week control period 1 of the following diets in both of 2 cups: (1) standard diet,[2] (2) standard diet plus flavor, (3) standard diet plus vitamin, (4) standard diet plus vitamin plus flavor. Other vitamins were fed separately as pills.[3] During this 3-week period, the cups were alternated in a predetermined random manner, and the amount of food eaten from each cup was recorded daily. At the end of the period,

[2] The standard diet consisted of 24% "Labco vitamin-free" casein, 10% "Primex," 62% sucrose, and 4% salt mixture (Jones and Foster, '42). Flavored diets contained 10 p.p.m. oil of anise. Vitamin-containing diets contained 5 μg/gm vitamin in the case of thiamine and pyridoxine, and 10 μg/gm in the case of riboflavin.

[3] One pill was given each rat daily. It contained approximately: 60 μg thiamine hydrochloride, 120 μg riboflavin, 90 μg pyridoxine hydrochloride, 150 μg calcium pantothenate, 10 mg choline chloride, 1 mg α-tocopherol, and 55 I.U. vitamin A, and 11 I.U. vitamin D as 0.001 ml Natola, all in a dextrin-powdered sugar base. In the thiamine experiment, thiamine was omitted from the pills; in the riboflavin experiment, riboflavin was omitted; in the pyridoxine experiment this vitamin was omitted.

half of the animals were suffering from severe vitamin deficiency while the other half were considered to be normal.

The 3-week experimental period was broken into 4 parts, during which half of the deficient animals received choices shown in column (1) of table 1 and half received the choices shown in column (2). Similarly, half of the normal animals received the choices in column (1) and half those in column (2). The cups were alternated as before and the amount of food eaten from each cup recorded daily.

TABLE 1

Plan of experimental period.

PART	LENGTH OF PERIOD DAYS	CHOICES	
		(1)	(2)
First	5	Unflavored, no vitamin; flavored, vitamin-containing	Flavored, no vitamin; unflavored, vitamin-containing
Second	5	Flavored, no vitamin; unflavored, vitamin-containing	Unflavored, no vitamin; flavored, vitamin-containing
Third	5	Same as first period	Same as first period
Fourth	6	Same as second period	Same as second period

RESULTS

As was anticipated from previous experiments, at the start of the experimental period in all 3 experiments the deficient animals ate the vitamin-containing diet primarily, while the normal animals showed little preference. In the second part of the experimental period, the deficient animals ate chiefly of the vitamin-free diet, which was now flavored the same as the vitamin-containing diet had been in the first part of the experimental period. Normal animals for the most part again showed little distinctive preference. In the third part, choice was similar to what it had been in the first part, while in the last part, the results were variable.

The data are presented in tables 2 and 3, while the marked alternation of the amount of vitamin-containing diet eaten

TABLE 2

General analysis of results.[1]

GROUP	CONTROL PERIOD		EXPERIMENTAL PERIOD			
	Weight gain	Food eaten	Weight gain	Food eaten	Change in per cent eaten from cup I [2]	Per cent eaten associated with flavor [3]
	gm	gm	gm	gm		
			Thiamine			
Deficient	14.1 ± 3.2	95.6 ± 4.2	71.1 ± 4.5	194.3 ± 14.3	17.2 ± 5.3	70.3 ± 7.0
Normal	74.8 ± 2.2	166.4 ± 5.4	69.6 ± 6.6	286.6 ± 14.3	—2.8 ± 4.4	49.5 ± 7.5
			Riboflavin			
Deficient	10.2 ± 1.8	79.2 ± 5.3	70.2 ± 4.1	175.1 ± 7.9	25.4 ± 4.2	63.9 ± 3.6
Normal	67.0 ± 3.8	156.8 ± 9.0	62.9 ± 5.3	228.0 ± 14.9	12.5 ± 4.1	45.5 ± 5.6
			Pyridoxine			
Deficient	18.4 ± 2.3	92.3 ± 5.4	65.8 ± 2.7	169.3 ± 6.9	22.3 ± 6.5	67.2 ± 6.5
Normal	59.2 ± 2.9	134.6 ± 6.9	53.1 ± 3.8	205.1 ± 4.4	1.3 ± 6.9	55.7 ± 4.4

[1] All results in terms of the mean and standard of the mean.

[2] Control period minus experimental period. The vitamin-containing food was in all cases in cup II in the experimental period, and thus a positive change in per cent eaten from cup I indicates an overall preference for the vitamin-containing food.

[3] Per cent eaten of that food, flavored or unflavored, which first contained the vitamin in the experimental period. A result significantly greater than 50 indicates an association of flavor with beneficial effect of the vitamin.

TABLE 3

Detailed analysis of experimental period.

GROUP	FIRST PART		SECOND PART		THIRD PART		FOURTH PART	
	Weight gain	Per cent eaten [1]	Weight gain	Per cent eaten	Weight gain	Per cent eaten	Weight gain	Per cent eaten
	gm/day		*gm/day*		*gm/day*		*gm/day*	
				Thiamine				
Deficient	3.18 ± .51	94.0 ± 3.2	2.46 ± .64	35.8 ± 11.5	3.54 ± .40	85.3 ± 8.2	4.20 ± .28	46.5 ± 11.9
Normal	3.48 ± .24	36.8 ± 7.6	3.62 ± .34	46.8 ± 9.1	3.08 ± .36	54.9 ± 8.7	2.95 ± .37	47.6 ± 6.6
				Riboflavin				
Deficient	4.36 ± .42	79.5 ± 9.0	3.22 ± .30	46.1 ± 8.1	3.32 ± .36	90.2 ± 5.0	2.62 ± .48	58.6 ± 6.8
Normal	2.74 ± .48	46.7 ± 12.3	3.80 ± .44	79.7 ± 6.9	2.86 ± .28	70.1 ± 8.5	2.48 ± .30	55.0 ± 7.5
				Pyridoxine				
Deficient	4.28 ± .32	78.1 ± 10.5	2.58 ± .28	35.9 ± 12.8	3.50 ± .22	90.3 ± 6.2	2.33 ± .25	63.4 ± 7.3
Normal	3.14 ± .26	57.2 ± 8.0	2.56 ± .24	56.2 ± 9.3	2.42 ± .20	56.3 ± 9.6	2.08 ± .23	39.7 ± 4.7

[1] Per cent of food eaten from the diet containing the vitamin.

by deficient animals with change in flavor is shown in figure 1. It should be noted from table 3 that the deficient animals failed to gain as much weight during the second part of the experimental period when their choice of diet with respect to the vitamin was poor as they did during the first and third parts when they ate primarily the vitamin-containing diets. From table 2 it is seen that the deficient animals had significant tendencies both to eat that diet, flavored or unflavored, which had contained the vitamin at the start of the experi-

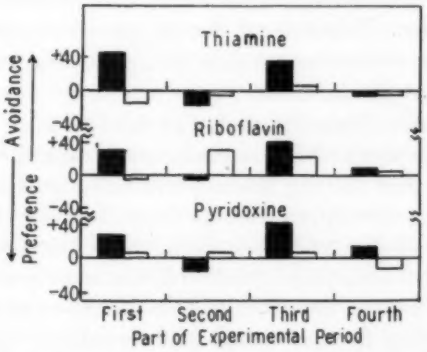

Fig. 1 Effect of alternation of flavor on appetite for vitamin-containing foods. Flavor was alternated with respect to the vitamin between each part of the experimental period. Preference (positive) or avoidance (negative) was calculated as per cent eaten of vitamin-containing food minus 50. Solid bars — average of 10 deficient animals; open bars — average of 10 normal animals.

mental period, and to eat more of the vitamin-containing diet than of the vitamin-free diet. Further analysis of the data indicated that there was no preference or avoidance of anise flavor as such and that there were no evident sex differences in eating behavior.

DISCUSSION

The alternation in amount eaten of vitamin-containing diet with change in flavor shown by deficient animals in figure 1, and the significant tendency of these animals to eat more of the diet of that flavor originally associated with the vitamin,

are proof of an association by the deficient animals of some deficiency-relieving quality of the vitamin with flavor or lack of it. This association must have involved a learning process, since flavor or lack of it was an artificial, not an inherent, characteristic of the vitamin. Thus, the appetites shown by rats for thiamine, riboflavin, and pyridoxine appeared to be learned appetites as proposed by Harris et al. ('33), and not true hungers. Harris concluded that the production of a feeling of well-being in the deficient animal was that quality of the vitamin-containing diet which was responsible for the animals' choice. This was, of course, an assumption, since one cannot easily determine what a rat feels. No other explanation seemed probable, however.

As is evident from figure 1, the tendency of the deficient animals to eat more of the vitamin-containing diet than of the vitamin-free diet during the experimental period as a whole was caused by incomplete change from the vitamin-containing diet to the vitamin-free diet when the flavors were switched after the first and third parts of the experimental period. The rats did not eat the vitamin-free diet to as great an extent nor for as long in the second and fourth 5-day periods as they had eaten the vitamin-containing diet in the 2 previous 5-day periods. This was not primarily due to an association of the effects of the vitamin with something other than flavor (e.g., flavor of the vitamin itself), because the eating behavior of only 3 out of the 30 deficient animals used in these experiments could possibly be interpreted in this light (i.e., only 3 animals did not exhibit at least a slight tendency to associate the vitamin with flavor or lack of it). Nor was it due to a relearning process, in which the vitamin became associated with the new flavor in the second and fourth periods, because when the original choices were offered in the third part of the experimental period, the preferences for the vitamin-containing diet were as strong as or stronger than in the first part.

The explanation was to be found primarily then in lack of stimulus to eat the vitamin-free diet. There must be 2 reasons

why deficient animals in the usual experiment continue to have an appetite for the vitamin-containing diets. One is that a stimulus to eat it (presumably the derived feeling of well-being) exists over a period of time; and in these experiments it must have existed over a period of at least 15 days. The other is that a habit is set up which makes customary the eating of a diet with a particular recognizable physical characteristic — in this case, flavor, or lack of it. A clear explanation of our results is then possible. During the first and third parts of the experimental period, both stimulus and habit contrived toward the eating of the vitamin-containing diet, but during the second and fourth parts habit exerted its influence in favoring choosing of the vitamin-free diet but stimulus was lacking. Consequently, the shift from vitamin-containing to vitamin-free diets when flavor was switched the first and third times was incomplete.

It has been found in 2 experiments on riboflavin deficiency, 1 of which is reported here, that animals previously receiving riboflavin have a slight but definite appetite for riboflavin. This appetite was significantly less obvious than that found in animals previously fed a diet deficient in riboflavin, and it was not apparent in our first published experiment on riboflavin (Scott and Quint, '46b). This appetite accounts for the unusual behavior of the normal group in the riboflavin experiment when compared with the normal groups in the thiamine and pyridoxine experiments. During the second and third parts of the experimental period, the normal animals preferred the riboflavin-containing diet to a certain extent, but there was no evidence of an association by this group of preference for riboflavin with flavor.

SUMMARY

The data obtained in these experiments are interpreted as meaning that the appetites for thiamine, riboflavin, and pyridoxine shown by animals previously fed diets deficient, respectively, in these vitamins are learned, probably as a result of beneficial experience. It is suggested that in the

usual experiment, deficient animals continue to eat a vitamin-containing diet for 2 reasons: (1) A habit is set up involving association of a feeling of well-being with some characteristic of the diet; and (2) a stimulus to eat the diet, presumably the derived feeling of well-being, persists over a considerable period of time. Animals previously fed a diet containing riboflavin showed a slight but definite preference for a riboflavin-containing diet.

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THE CORNEAL VASCULARIZATION RESULTING FROM DEFICIENCIES OF AMINO ACIDS IN THE RAT [1]

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NINE FIGURES

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The association of corneal vascularization in man with nutritional deficiencies has led to a study of this phenomenon in experimental animals. Among the various deficiencies which will result in corneal vascularization in the rat are protein deprivation (Hall et al., '46) and deficiencies of lysine, tryptophane (Totter and Day, '42), methionine (Berg and co-workers, '47), leucine and histidine (Maun, Cahill and Davis, '45b, '46). In phenylalanine deficiency Maun, Cahill and Davis ('45a) found that "neither the eyes of the deficient, nor those of the control animals, showed alterations." Sydenstricker and associates ('46) have suggested that corneal vascularization may be a reaction resulting from a deficiency of any of the indispensable amino acids, or of protein. It thus seemed desirable to determine whether deficiencies of other amino acids resulted in changes in the cornea.

METHODS

Twenty-five or 26-day-old rats of a Wistar strain were placed in individual cages and given a control diet or one de-

[1] A preliminary report of this work was published in the Proc. Soc. Exp. Biol. Med., *64:* 59, 1947.

ficient in phenylalanine, isoleucine, leucine, arginine, histidine, threonine or valine. The details of the methods and techniques used and the care of the animals were as previously described (Bowles et al., '46). As a source of protein in the control diet, the amino acid mixture of Maun, Cahill and Davis ('45a) was used, which supplied approximately 3 times the minimum required amount of each of the indispensable amino acids in utilizable form. The amino acid mixture used in the deficient diets was identical with this except that in each deficient diet the appropriate amino acid in the mixture was replaced with sucrose. The remainder of each diet contained in 100 gm: salt mixture [2] 4 gm, cod liver oil 2 gm, cottonseed oil 3 gm, choline chloride 0.2 gm, riboflavin 1.6 mg, thiamine hydrochloride 0.4 mg, calcium pantothenate 2 mg, pyridoxine hydrochloride 0.4 mg and sufficient sucrose to make 100 gm.

Para-aminobenzoic acid and inositol were not included in the diet since they appear to favor synthesis of amino acids by the intestinal flora (Hall and Sydenstricker, '47). The corneas of rats fed control diets to which were added only the vitamins as used in the present study showed no significant abnormality either on examination with the biomicroscope or in histological sectioning (Berg et al., '47).

One litter of 8 rats, 8 litters of 6 rats each, 3 litters of 5 rats, 2 litters of 4 rats and 1 litter of 3 rats were distributed among the diets so that the number of rats on each diet was as shown in table 1. No 2 rats from any litter were on the same diet.

In an attempt to produce more extensive corneal changes in rats deficient in threonine and valine, 3 rats were placed on each of 3 diets which were similar to the control diet except that the first contained only 0.30% of DL-valine, the second 0.20% of DL-threonine and the third 0.35% of DL-threonine. One rat from each group where corneal vascularization occurred was changed to the control diet to induce regression of vascularization. Two or 3 rats from these groups having

[2] The salt mixture was the same as that used by McKibben, Madden, Black and Elvehjem ('39).

marked corneal vascularization typical of the group as shown by biomicroscopic examination were selected for injection and subsequent photography of the cornea. Biomicroscopic examinations of the rats' eyes were made 4 times weekly with most of the rats and 3 times weekly for the remainder.

As before, we limit our use of the term "corneal vascularization" to the situation where there is an actual development of capillaries in the cornea beyond the limits of normal variation.

TABLE 1

Corneal vascularization and growth of experimental rats.

DIET	NO. OF RATS IN GROUP	INCIDENCE OF CORNEAL VASCULARIZATION	DAYS REQUIRED FOR APPEARANCE OF CORNEAL VASCULARIZATION		AVERAGE CHANGE IN WT. PER DAY DURING 30 DAYS
			Range	Average	
					gm
Control	8	0			+ 1.2
Phenylalanine-deficient	11	11	3–13	7	— 0.6
Leucine-deficient	11	10	3–19	11	— 0.6
Isoleucine-deficient	11	11	4–11	8	— 0.7
Arginine-deficient	8	7	4–20	11	+ 0.7
Histidine-deficient	13	11	4–34	15	— 0.3
Threonine-deficient	11	9	3–36	19	— 0.5
Valine-deficient	9	9	3–20	11	— 0.8

Such normal variation is remarkably slight. An occasional rat, certainly less than 1 in a hundred, will have scleral flaps carrying aberrant portions of the limbic plexus which extend slightly beyond the usual periphery of the cornea. Even less frequently, accidental trauma to a cornea may cause confusing corneal changes.

RESULTS AND DISCUSSION

Quite regularly, the first sign of ocular reaction to deficiency of amino acids or of protein is congestion of the scleral conjunctiva and engorgement of the limbic plexus. Soon thereafter, often concurrently, there is slight thickening and diffuse clouding of the cornea, probably due to edema. Subsequently,

short capillary "sprouts" can be seen which derive from the marginal vessels of the limbic plexus. It is thought that these actually are the afferent limbs of small capillary loops. Such "sprouts" increase in length and caliber and soon their efferent limbs are visible. As such capillary loops invade the subepithelial region of the cornea, anastomotic vessels are apt to develop so that the entire cornea may become shrouded in a dense network of centripetally growing vessels in which no definite pattern can be made out.

Corneal opacity due to edema and leukocytic infiltration tends to parallel the severity of vascular invasion. At times, diffuse opacity may prevent accurate evaluation of the degree of vascularity present. In some animals scattered nebular opacities precede the general clouding. The grade of vascularization, its pattern and the severity of the associated opacity vary much in different deficiencies. With deficiency of total protein, or of phenylalanine, leucine or isoleucine, corneal vascularization and opacity may be extreme so that the tissue seems to be converted into an area of granulation tissue covered by finely wrinkled, dry, sometimes ulcerated, epithelium. This we have also observed to be the result of tryptophane deficiency.[2] In deficiency of histidine, valine, or threonine the invading vessels remain small and their radial arrangement seldom is disturbed. Corneal opacity may be very slight so that the newly formed vessels are visualized with little difficulty and no abnormality of the corneal epithelium can be seen. This is similar to what we have observed to occur in methionine deficiency (Berg et al., '47). Rats deficient in phenylalanine and lysine [3] have shown some variation in the grade of keratitis they developed but extremes have not been observed.

The rats fed diets devoid of valine and threonine usually died as early corneal changes appeared. In the 3 rats fed 0.30% of DL-valine the rats lived long enough to develop more extensive corneal changes and vascularization. This diet supplied 0.15% of L-valine as compared to the 0.7% of

[3] Unpublished data.

this form of valine required for growth (Rose, '37). The rats fed 0.35% and those fed 0.20% of DL-threonine developed congestion of the sclera and a slight degree of corneal opacity but no corneal vascularization. These 2 groups of rats received, respectively, 0.17% and 0.10% of L-threonine, the form utilizable for growth, as compared to the rats' requirement of 0.5% for growth (Rose, '37).

The rats in the group fed the arginine-deficient diet developed only a very minor degree of corneal vascularization, which later spontaneously regressed. One of the control rats developed a few capillary loops into the edge of the cornea beyond the limits of normal variation. The corneas of the remaining control rats appeared normal except for an occasional slight translucency of the cornea which would later disappear. Maun, Cahill and Davis ('45a) noted no histological changes in the eyes of their control rats fed a diet similar to this.

When the deficient rats were returned to the control diet to supply the lacking essential amino acid, prompt regression of the vascularization occurred until corneal capillaries could no longer be seen with the biomicroscope. The time required for complete regression varied somewhat, depending on the degree of corneal vascularization.

The incidence and time of appearance of vascularization in the groups of rats and growth data are given in table 1. It may be seen that vascularization appeared most rapidly in phenylalanine deficiency and most slowly in threonine deficiency.

All the rats on the deficient diets lost weight, except for the arginine-deficient rats which grew at a rate of 0.7 gm per day. The growth of 1.2 gm per day for the control rats may be compared with the rate of about 3.0 gm per day usual for our strain of rats at this age when receiving an adequate diet. Since the protein requirement of the rats was supplied with amino acids, a subnormal growth would be expected, as this diet would lack the growth factor for rats described by Womack and Rose ('46) as present in intact and partially

hydrolyzed proteins. Taking into consideration the difference in rate of normal growth of the strains of rats used by Womack and Rose ('46) and those used in the present study, the diets used by Womack and Rose, which contained the non-essential amino acids, resulted in considerably better growth than did our control diet which lacked these amino acids. With the chick, Luckey and associates ('47) obtained superior growth with diets containing some of the non-essential amino acids as compared with diets containing only the amino acids now recognized as essential for the chick.

Plates 1 and 2 show reproductions of photographs of injected corneas illustrating the type of vascularization obtained in each of the deficiencies studied. It will be noted that while there are differences in pattern of vascularization in the deficiencies studied here, and in those previously studied, these differences in no case are very striking and the patterns of vascularization in several of the deficiencies are indistinguishable. The observations which we made in earlier studies as to development of pattern of vascularization also apply here.

The appearance of enlarged distal ends of developing capillaries in threonine, leucine and phenylalanine deficiencies is similar to what we had observed before in protein deprivation. The pictures of injected corneas here may be compared with the pictures of the injected corneas of normal rats shown in an earlier paper (Bowles et al., '46).

Our observations that phenylalanine deficiency will cause corneal vascularization are not in agreement with the statement of Maun, Cahill and Davis ('45a) that they observed no alteration of the eyes of their phenylalanine-deficient animals. It is possible that this discrepancy may be explained as being a strain difference since Maun, Cahill and Davis used Sprague-Dawley animals while rats of a Wistar strain were used in the present study.

SUMMARY

Deficiencies of phenylalanine, isoleucine, threonine, valine and arginine were found to result in corneal vascularization.

The corneal vascularization resulting from leucine and histidine deficiencies was also studied. It now seems clear that lack of protein or of any 1 of the 10 essential amino acids may result in corneal vascularization in the rat. Other changes observed in varying degrees were diffuse and nebular corneal opacity, and edema of the cornea.

ACKNOWLEDGMENT

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PLATE 1

EXPLANATION OF FIGURES

(All 18 × reduced approximately one-third)

Oblique views of India ink-injected corneas of rats fed amino acid-deficient diets.

1 Histidine-deficient diet. Corneal vascularization observed after 16 days, rat killed after 33 days on the diet.

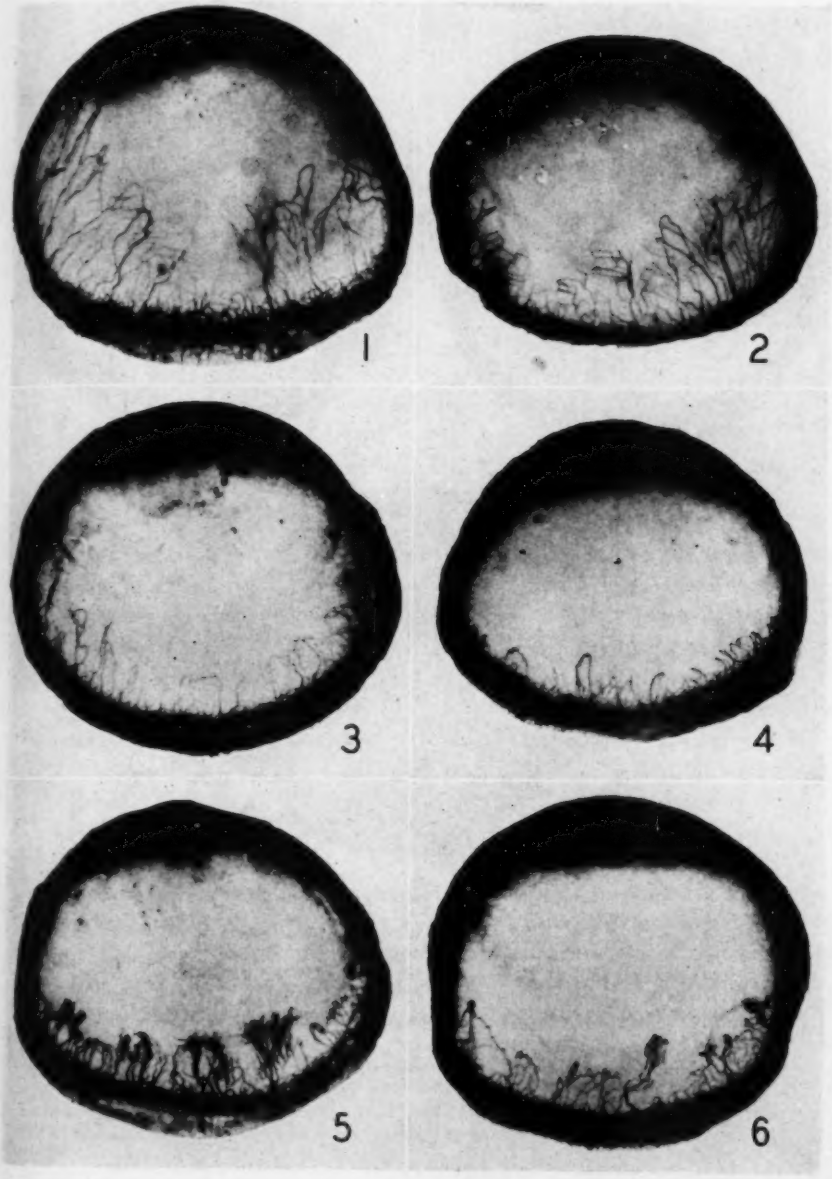
2 Histidine-deficient diet. Corneal vascularization after 16 days, rat killed after 33 days on the diet.

3 Low-valine diet (0.30% of DL-valine). Corneal vascularization after 13 days, rat killed after 54 days on the diet.

4 Valine-free diet. Corneal vascularization after 19 days, rat killed after 23 days on the diet.

5 Threonine-deficient diet. Corneal vascularization after 25 days, rat killed after 46 days on the diet.

6 Isoleucine-deficient diet. Corneal vascularization after 11 days, rat killed after 49 days on the diet.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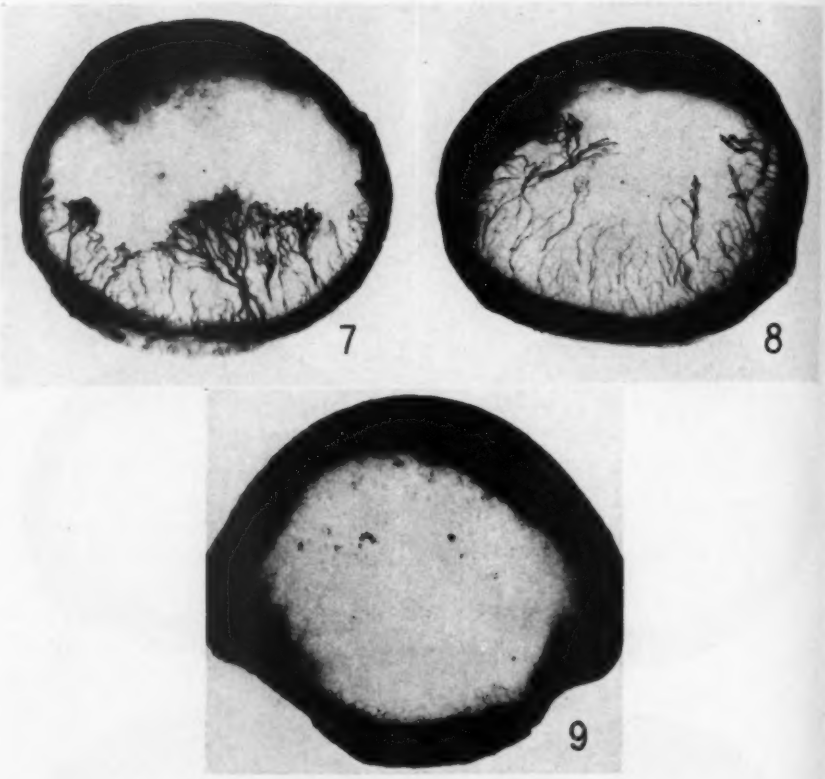

(All 18 × reduced approximately one-third)

Oblique views of India ink-injected corneas of rats fed amino acid-deficient diets.

7 Leucine-deficient diet. Corneal vascularization observed after 8 days, rat killed after 29 days on the diet.

8 Phenylalanine-deficient diet. Corneal vascularization after 5 days, rat killed after 51 days on the diet.

9 Arginine-deficient diet. Corneal vascularization after 4 days, rat killed after 81 days on the diet.

THE PROTEIN REQUIREMENTS OF THE ADULT RAT IN TERMS OF THE PROTEIN CONTAINED IN EGG, MILK AND SOY FLOUR[1]

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ONE FIGURE

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Recently several studies have been reported of the protein value of foods for mature animals using a method proposed by Melnick and Cowgill ('37) and demonstrated with dogs. The method involves the use of animals in negative nitrogen balance at various levels of nitrogen intake, and receiving adequate caloric intakes. Under such conditions the relationship between nitrogen intake (or absorbed nitrogen) and nitrogen balance is a linear one. The quality of the protein as well as the quantity needed to maintain nitrogen equilibrium can be estimated from the mathematical description of the regression line describing the relationship. This method was used by Harris and Mitchell ('41) to study the utilization of urea nitrogen in sheep and was also employed by Allison and Anderson ('45) to study the quality of various proteins fed to dogs. The method was adapted to human subjects in a study of protein requirements in terms of different foods by Bricker, Mitchell and Kinsman ('45) and later, in a modified form, by Hegsted and coworkers ('46) who applied it to mixed diets. The present report deals with an application of this method to adult male rats.

[1] The data reported in this paper were taken in part from a thesis presented by Mildred Bricker to the Graduate School of the University of Illinois in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

EXPERIMENTAL PROCEDURE

Ten adult male albino rats were fed low-protein diets at 2 levels of intake in amounts sufficient to maintain body weight and energy equilibrium. The food protein sources, dried defatted whole egg, dried skim milk and soy flour (low-fat, expeller process) were each fed at one level estimated to give a slightly negative nitrogen balance and then at a lower level.

TABLE 1

Composition of experimental diets.

	DIET A	DIET B	DIET C	DIET D	DIET F	DIET G
Starch	31.28	29.91	27.21	22.86	23.57	19.81
Cerelose	40.00	40.00	40.00	40.00	40.00	40.00
Vitamin supplement [1]	5.00	5.00	5.00	5.00	5.00	5.00
Mineral salts	4.00	4.00	4.00	4.00	4.00	4.00
Sodium chloride	1.00	1.00	1.00	1.00	1.00	1.00
Wood flock	4.00	4.00	4.00	4.00	4.00	4.00
Lard	9.65	9.48	9.94	9.90	9.26	8.96
Wheat germ oil	0.50	0.50	0.50	0.50	0.50	0.50
Cod liver oil	1.50	1.50	1.50	1.50	1.50	1.50
Barium sulfate	1.00	1.00	1.00	1.00	1.00	1.00
Dried defatted whole egg	3.07	4.61	...	...	...	...
Dried skim milk	...	...	5.85	10.24	...	...
Soy flour [2]	...	...	...	...	10.17	14.23
Total	101.00	101.00	100.00	100.00	100.00	100.00
Percent protein (N × 6.25)	2.38	3.38	2.38	4.02	5.15	7.14

[1] The vitamin supplement added in a 5% starch vehicle provides in 100 gm of the diet: 500 μg thiamine, 650 μg riboflavin, 300 μg pyridoxine, 3 mg pantothenic acid, 400 mg choline, 60 mg nicotinamide and 500 μg ascorbic acid.

[2] Low-fat expeller process soy flour obtained directly from the A. E. Staley Manufacturing Company, Decatur, Illinois.

For the egg protein, diets containing higher levels were employed, designed for each rat, on the basis of the data secured on the 2 lower levels, to support approximate nitrogen equilibrium. The composition of the diets, with the exception of the latter, may be found in table 1. An attempt was made to have all the diets similar and adequate in nutritive value in all respects except protein. The total amount of fat was kept at

12%, including the fat in the test foods. Barium sulfate was used as a filler so that on days when feces markers were given the barium sulfate could be replaced with ferric oxide.

The quantity of diet given each rat was sufficient for energy equilibrium as measured in a pre-feeding period of 11 days by maintenance of constant body weight. On each diet a 5-day preliminary adjustment period was followed by a 7-day collection period. Urine samples were preserved with dilute hydrochloric acid and pooled for the entire week. The week's feces samples were composited, dried at 110°C. and ground before analysis. Nitrogen analyses were made on the food and excreta by the macro-Kjeldahl method, using mercury as a catalyst.

EXPERIMENTAL RESULTS

The essential data from the metabolism studies with whole egg protein fed at the various dietary levels are summarized in table 2. The food intakes of the rats were essentially constant from period to period. With increasing levels of nitrogen intake, the fecal nitrogen per gm of food showed no significant change, averaging 1.21 mg. This circumstance shows that the nitrogen of the whole-egg preparation used in these experiments was completely digestible and that the fecal nitrogen is all of body (metabolic) origin, confirming the observations of Mitchell and Carman ('26b).

The daily urinary nitrogen per unit of metabolic mass ($W^{\frac{2}{3}}$ gm) actually decreased with successive increases in nitrogen intake, averaging 0.826, 0.737 and 0.692 mg, respectively. The nitrogen balances become less negative and, eventually, positive. Comparing the average changes in nitrogen intake with the average change in nitrogen balance, it appears that an increase of 1 mg of nitrogen consumed is associated with a decrease in the loss of nitrogen to the body of 1.28 mg in passing from the lowest to the intermediate level of dietary protein, and of 1.38 mg of nitrogen in passing from the intermediate to the highest level of dietary protein. This anomalous relationship probably signifies a depression in the minimum

endogenous catabolism of nitrogen with continued experimentation involving prolonged periods of nitrogen depletion, analogous to the depression in the basal metabolism after long periods of inanition.

That this is not a specific effect of egg protein is indicated by the results of an earlier experiment on young rats. Two groups of 4 rats each were fed alternately, in successive (and

TABLE 2

The nitrogen metabolism of the rats on 3 levels of whole egg proteins.

RAT NO.	FECAL NITROGEN PER GM OF FOOD CONSUMED			URINARY NITROGEN PER $W^{\frac{2}{3}}$ GM			DAILY NITROGEN BALANCE		
	2.38% protein [1]	3.28% protein	3.84% protein [2]	2.38% protein	3.28% protein	3.84% protein [2]	2.38% protein	3.28% protein	3.84% protein [2]
	mg	*mg*	*mg*	*mg*	*mg*	*mg*	*mg*	*mg*	*mg*
1	1.20	1.09	1.09	0.87	0.63	0.60	—29	+ 6.8	+17
2	1.01	1.11	1.17	0.83	0.71	0.73	—22	+ 0.5	+ 9
3	1.19	1.26	1.20	0.75	0.64	0.63	—21	+ 2.8	+14
4	1.15	1.18	1.22	0.71	0.66	0.66	—17	+ 1.8	+11
5	1.03	1.10	1.16	0.71	0.76	0.69	—15	— 2.8	+14
7	1.22	1.28	1.09	0.72	0.67	0.58	—21	— 0.3	+20
8	1.36	1.33	1.11	0.74	0.66	0.61	—28	— 1.7	+20
11	1.17	1.26	1.34	1.00	0.97	0.83	—34	—17.1	+20
12	1.37	1.40	1.29	0.94	0.83	0.90	—37	—10.9	—0.4
14	1.33	1.31	...	1.00	0.85	...	—43	—10.7	..
Averages	1.20	1.23	1.19	0.826	0.737	0.692	—26.7	— 3.1	+13.9

[1] In these headings, the term protein refers to conventional protein = N × 6.25. The metabolism tests were carried out from July 9 to 16, July 23 to 29 and August 29 to September 4, respectively.

[2] The diets used in this period varied in protein level from rat to rat from 3.59 to 4.62% (averaging 3.84), in an attempt to approximate the level required for nitrogen equilibrium.

continuous) 7-day metabolism periods, a 4% egg-protein diet and a nitrogen-free diet. The order of alternation differed in the 2 groups. The data are summarized in table 3. The rate of decline in urinary nitrogen per $W^{\frac{2}{3}}$ gm is again evident, but it is not appreciably affected by the order of feeding of the experimental diets. Specifically, the individual differences in the 2 groups of rats between the observed value for the

second period and the mean for periods 1 and 3 were not statistically distinct (P = ca. 0.27).

This experiment shows also that the nitrogen of whole egg is completely utilized in the metabolism of the growing rat within the limits of accuracy of the methods used, confirming again the results of Mitchell and Carman ('26b). That this is also true for the adult male rat is demonstrated by the

TABLE 3

The utilization of the nitrogen of whole egg by the growing rat. Successive 7-day collection periods.

DIET	NO. OF RATS	AVERAGE BODY WEIGHT	DAILY FOOD AVERAGE	AVERAGE FECAL N PER GM OF FOOD	AVERAGE URINARY N PER $W^{\frac{3}{4}}$ GM	AVERAGE URINARY N PER $W^{\frac{3}{4}}$ GM FOR PERIODS 1 AND 3
		gm	*gm*	*mg*	*mg*	*mg*
Nitrogen — Low	4	57.1	3.34	1.74	1.138	
4% egg protein	4	55.6	3.39	1.98	.853	.910
Nitrogen — Low	4	52.7	2.62	1.41	.683	
4% egg protein	4	60.8	3.36	2.13	1.057	
Nitrogen — Low	4	56.1	3.25	1.44	.780	.888
4% egg protein	4	55.6	2.68	1.77	.719	

data collected in tables 4 and 5, taken from unpublished experiments in this laboratory (Treichler, '39). Both groups of rats subsisted for 14 days on the 4% egg-protein diet, collections of excreta being made for the last 7 days only. In this period the daily urines were composited for analysis. The rats were then put upon a nitrogen-free diet in which the dried defatted whole egg was replaced by sucrose, and the daily urine collections were analyzed separately with the results shown in the tables. All diets contained 22% of fat and approximately 4.6 cal. of gross energy per gm.

The rats yielding the data in table 4 averaged about 240 gm in weight. Their urinary nitrogen output per day was not significantly different on the 2 diets. Expressed per $W^{\frac{3}{4}}$ gm per day, the averages were 0.786 mg of nitrogen on the low egg-protein diet and 0.727 mg on the nitrogen-free diet. A

TABLE 4

The utilization of the nitrogen of whole egg at low levels of intake by young mature rats at 28°C. on maintenance rations.[1]

DAYS ON TEST	AVERAGE DAILY N INTAKE	DAILY EXCRETION OF URINARY NITROGEN				
		Rat 55	Rat 58	Rat 61	Rat 64	Rat 67
	mg	*mg*	*mg*	*mg*	*mg*	*mg*
		4% egg-protein diet				
7	52	52	41	46	55	47
		Low-nitrogen diet				
1	5	53	42	46	46	36
2	5	55	46	49	53	36
3	5	49	44	42	41	44
4	5	..	..	38	40	40
5	5	..	..	48	37	39
6	5	..	..	43	44	41
7	5	..	..	47	39	46
8	5	..	..	36	38	42
9	5	..	..	52	35	46

[1] Taken from the thesis of Ray Treichler ('39).

TABLE 5

The utilization of the nitrogen of whole egg by older mature rats on maintenance rations at comfortable temperatures.[1]

DAYS ON TEST	AVERAGE DAILY N INTAKE	DAILY EXCRETION OF URINARY NITROGEN						
		Rat 29	Rat 30	Rat 31	Rat 32	Rat 33	Rat 34	Average for 6 rats
	mg	*mg*	*mg*	*mg*	*mg*	*mg*	*mg*	*mg*
		4% egg-protein diet						
7	64	77	70	70	86	72	58	72
		Low-nitrogen diet						
1	5	63	49	47	56	65	50	55
2	5	70	46	61	54	61	61	59
3	5	75	50	50	63	61	53	59
4	5	85	51	90	57	62	62	68
5	5	68	54	53	60	74	49	60
6	5	75	57	65	59	55	49	60
7	5	65	42	55	54	60	51	54

[1] Taken from the thesis of Ray Treichler ('39).

statistical analysis of the differences for individual rats afforded no basis for suspecting a diet effect. The rats were in negative nitrogen balance in both periods.

The second group of rats (table 5) averaged 390 gm in weight, and here the urinary nitrogen on the egg-protein diet averaged higher for all 6 rats than that on the low-nitrogen diet, 0.821 mg per $W^{\frac{3}{4}}$ gm against 0.680 mg. The probability that a fortuitous combination of the uncontrolled factors in the experiment could have produced an average difference as large or larger than this, and in the same direction, is only 0.016 (Student, '08) and may be neglected. However, in this experiment all rats were in positive nitrogen balance on the egg-protein diet, averaging 19.7 mg per day, so that the urine may have contained nitrogen consumed in excess of body needs.

The experimental data in these 2 tables bring further support to the conclusion that whole-egg protein is utilized in the metabolism of the mature rat with little or no wastage (table 4), or at least they are not in contradiction necessarily with this conclusion (table 5). It is to be noted in particular that egg protein in no case depressed the urinary nitrogen level secured on a low-nitrogen diet, in contrast to the observations reported by Brush, Willman and Swanson ('47).

Since the data on fecal nitrogen presented in table 2 indicate clearly that the nitrogen of the preparation of dried defatted whole egg used in these tests is completely digestible, the metabolic nitrogen in the feces may be computed in tests with other proteins by using the factor 1.21 mg per gm of food. This procedure has been used in computing the true digestibility of the nitrogen of milk and of soy flour with results summarized in table 6. The average true digestibility of milk nitrogen for these mature rats was 95.3 and that for the soy flour nitrogen was 90.4.

On the basis of evidence already presented, a biological value of 100 was assumed for the egg proteins. Therefore, the average urinary nitrogen excretion on the first 2 egg periods was assumed to be the endogenous nitrogen. These

values may be expressed in relation to the basal metabolism, in order to compare them with those published by Smuts ('35). The surface area of each rat was, therefore, estimated by the Lee ('29) formula. A basal metabolic rate of 686 (S.D. 97) cal. per m^2 per 24 hours was then assumed. This figure is an average of 70 basal metabolism determinations found in the literature. In arriving at this figure, the surface areas of the rats were all computed, or recomputed, by the formula of Lee. This meant recalculation of the original values in

TABLE 6

The true digestibility of the proteins (nitrogen) in milk and in soy flour for mature rats.

RAT NUMBER	MILK PROTEINS		SOY FLOUR PROTEINS	
	True digestibility	Biological value [1]	True digestibility	Biological value [1]
	%	%	%	%
1	93	96	93	51
2	95	87	88	48
3	94	90	92	51
4	97	89	91	45
5	94	88	88	50
7	94	83	89	53
8	98	82	..	..
11	94	96	90	62
12	96	75	92	56
14	98	100	..	..
Averages	95.3	88.6	90.4	52.0

[1] Computed by assuming the endogenous nitrogen outputs in column 5 of table 7, and proceeding in accordance with the method of Mitchell ('24).

some cases. Only data for adult male rats were taken, including 24 basal metabolic rate determinations by Mitchell and Carman ('26a), 15 determinations by Smuts ('35), 26 by Benedict, Horst and Mendel ('32) and 5 by Treichler and Mitchell ('41).

Estimates of surface area and basal caloric expenditures of the rats in this experiment are tabulated in table 7. The endogenous nitrogen in the urine is expressed in mg per basal calorie, and averages for all 10 rats 2.162 ± 0.120. This value

agrees well with that of 1.988 ± 0.034 mg reported by Smuts ('35) for adult rats.

This experiment was designed on the assumption that a linear relationship exists in the mature rat between the absorbed nitrogen and the nitrogen balance within the area of negative nitrogen balances. In figure 1 this relationship is shown graphically. Values for individual animals are expressed in relation to basal calories in accordance with the

TABLE 7

The weight, surface area, basal calories, and endogenous nitrogen of the adult male rats used in these experiments.

RAT NO.	BODY WEIGHT	SURFACE AREA [1]	BASAL METABOLISM [2]	ENDOGENOUS NITROGEN	
	gm	*cm²*	*cal./day*	*mg/day*	*mg per basal cal.*
1	312	393	26.96	55.05	2.042
2	313	394	27.03	56.44	2.088
3	324	402	27.58	53.00	1.922
4	326	404	27.71	52.50	1.895
5	314	395	27.10	54.75	2.020
7	364	432	29.64	57.66	1.945
8	408	462	31.69	63.66	2.009
11	294	380	26.07	69.66	2.672
12	334	410	28.13	69.10	2.456
14	336	411	28.19	72.51	2.572
Average					2.162 ± 0.120

[1] Calculated by the Lee formula, $S_{cm^2} = 12.54\ W_{gm}^{.60}$

[2] Assuming a basal metabolic rate of 686 cal. per square meter per 24 hours.

Terroine ('27)-Smuts ('35) principle. Regression lines were then fitted to the data by the method of least squares. The equation, $Y = a + bX$ was used, in which Y represents the nitrogen balance, X the intake of absorbed nitrogen, *a* the intercept on the X axis, i.e., the amount of nitrogen excreted in the urine and feces when no nitrogen is consumed, and *b* the rate of increase in Y as X increases, or the biological value of the protein, all values being expressed per basal calorie.

The resulting equations are:

$$Y = 0.990\,X - 2.757 \text{ for egg proteins,}$$
$$Y = 0.857\,X - 2.727 \text{ for milk proteins, and}$$
$$Y = 0.494\,X - 2.711 \text{ for soy flour proteins.}$$

The biological values (the b constants) indicated by these equations, expressed as percentages, are 99 for egg proteins, 86 for milk proteins and 49 for soy proteins, slightly less than the values that may be computed for the individual rats on the assumption of a biological value of 100 for egg proteins. These individual values have been summarized in table 6.

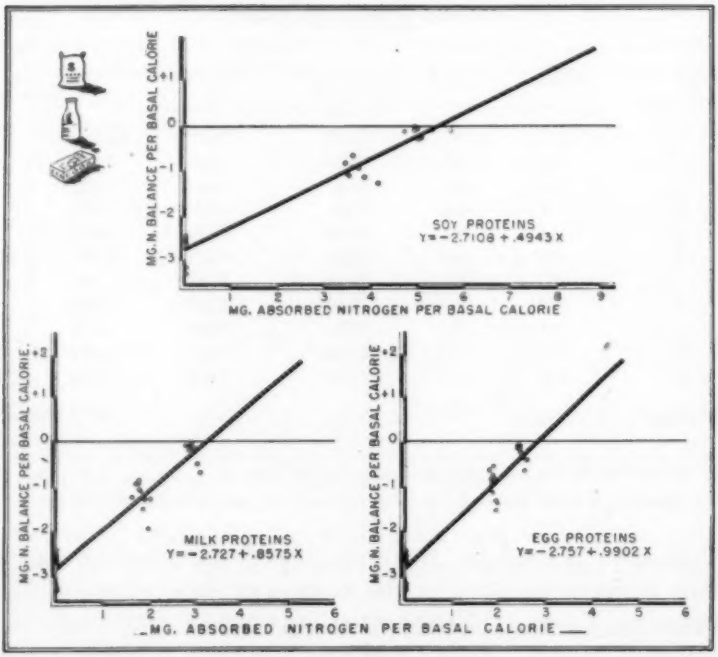

Fig. 1 The relationship between the truly absorbed nitrogen (X) and the nitrogen balance (Y) on the different experimental diets. In the equations given, the a value represents the nitrogen excretion at O intake and the b value, the slope of the line, or the biological value of the protein. The points at O intake are the average fecal plus urinary nitrogen excretion on the 2 low-egg periods.

The amount of absorbed nitrogen required for equilibrium may be calculated by solving for X when $Y = O$. Thus, in terms of truly absorbed nitrogen, 2.784 mg of egg nitrogen per basal calorie, 3.179 mg of milk nitrogen and 5.484 mg of soy-flour nitrogen are needed to maintain nitrogen equilibrium in the rats studied.

The amounts of total food nitrogen needed to maintain nitrogen equilibrium can be calculated by dividing the requirement of truly absorbed nitrogen by the true digestibility coefficient expressed as a decimal and are as follows: 2.784 mg of egg nitrogen per basal cal., 3.337 for milk and 6.066 for soy flour. When these nitrogen values are converted to the necessary per cents of protein in the diets used in this experiment they become 3.60 for egg, 4.33 for milk and 7.61 for soy proteins on the dry basis.

DISCUSSION

In similar studies by the authors (Bricker, Mitchell and Kinsman, '45) on human subjects a value of 2.76 mg of total milk nitrogen per basal cal. was needed for the maintenance of nitrogen equilibrium. This is to be compared with the value, on the same basis, of 3.34 mg for the rats in this study. If both requirements are computed on a weight basis, that for the human is 65 mg of nitrogen per kg and for the rat, 229. The agreement between the former values, as well as the disagreement between the latter is in harmony with the conclusions of Terroine ('27) and of Smuts ('35) that protein requirements for maintenance are related to surface area and basal metabolism rather than to body weight as is all too commonly assumed.

The corresponding values for soy-flour nitrogen are 2.88 mg per basal cal. for the human adult and 6.07 for the adult rat. The difference in this case is large. It may be due, but only in small part, to a difference in heat treatment of the soy flours fed to the 2 species. It is most likely largely traceable to a difference between the rat and man in the relative requirements for sulfur-containing amino acids that limit the bio-

logical value of soy flour proteins (Johnson et al., '47: Cox et al., '47), for the rat at least.

Barnes, Bates and Maack ('46), employing essentially the same technic as that used in this study, reported that mature male rats require for the maintenance of nitrogen equilibrium 11.2 mg of apparently absorbed egg nitrogen per day per 100 cm^2 of body surface and, for 2 different soy flours, 13.6 and 15.1 mg of apparently absorbed nitrogen. Using the Lee formula ('29) for the estimation of surface area rather than that of Carman and Mitchell ('26), and assuming a body weight of 325 gm, raises these requirements to 14.9, 18.1 and 20.1 mg N per 100 cm^2. The values obtained in this experiment are 19.0 mg of truly digestible egg nitrogen and 41.2 mg of truly digestible soy flour nitrogen per 100 cm^2 of body surface, or in terms of apparently digestible nitrogen, assuming 1.2 mg metabolic fecal N per gm of food (table 2), 15.0 mg egg N and 37.2 mg of soy flour N. The former value agrees well with the corresponding value of Barnes and associates, but the latter figure is considerably higher than the highest of the 2 values for soy flour reported by these investigators.

The biological value of 86 for milk protein agrees well with that of 86 determined by Boas-Fixsen and Jackson ('32) on 4 mature rats fed roller-process dried milk at a 7% protein level, and less well with that of 78 on mature rats fed at a 5% protein level by Sumner ('38). Sumner ('38) in the same study reports a value of 94 for egg proteins fed to mature rats at a 5% level of protein. Henry, Kon and Rowland ('46) obtained a biological value of 73 for milk protein for mature rats at an 8% level, a level too high to afford any assurance that dietary nitrogen is not wasted in metabolism merely because it is consumed in amounts exceeding requirements.

SUMMARY AND CONCLUSIONS

Ten adult male rats were fed 2 dietary levels each of milk and soy flour proteins and 3 dietary levels of egg protein; all of the former and 2 of the latter dietary levels were asso-

ciated with negative nitrogen balances. The linear relationship between truly absorbed nitrogen and nitrogen balance was described by regression equations fitted to the data by the method of least squares.

The amount of truly absorbed nitrogen (corrected for the metabolic nitrogen in the feces) required for nitrogen equilibrium was computed from these regression equations to be 2.78 mg of egg nitrogen, 3.18 mg of milk nitrogen and 5.48 mg of soy flour nitrogen per cal. of basal heat. The biological values indicated by these regression equations are 99 for whole egg nitrogen, 86 for milk nitrogen and 49 for soy flour nitrogen.

The nitrogen of the defatted dehydrated whole egg preparation used in these studies was completely digestible by the adult rat and, at the low levels of feeding used (2.38 to 4.62% conventional protein) the absorbed nitrogen was practically completely utilized in adult rodent metabolism. Neither in this experiment, nor in previous ones on adult rats carried out in this laboratory, has egg protein been observed to depress appreciably the minimum endogenous metabolism of nitrogen.

The total requirements of milk nitrogen per basal cal. for nitrogen equilibrium in the adult rat and the adult human are not greatly different, being 3.34 mg and 2.76 mg, respectively. The requirements of soy flour nitrogen on the same basis are much greater for the rat than for the human, 6.07 mg and 2.88 mg, respectively, testifying to the greater relative requirement of the rat than of the human for the amino acid severely limiting the nutritive value of soybean protein, namely, methionine (or cystine).

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A COMPARISON OF THE UTILIZATION AND ACCEPTABILITY OF FRESH AND DEHYDRATED FOOD[1]

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The increasing use of dehydrated foods, particularly during World War II, would seem to make desirable a complete study of their nutritive value. The prolonged periods over which such foods may be stored without spoilage as compared with fresh foods make them especially adaptable for army use. This property also is of importance in the use of dehydrated foods for relief overseas. The lowered bulk combined with the great decrease in weight of the water-free food makes the shipment of much larger amounts of food possible with a limited amount of shipping space.

[1] This work was done under a contract between the University of Southern California and the Research and Development Branch, Military Planning Division of the Office of the Quartermaster General, U. S. A.

The subject matter of this paper has been undertaken in cooperation with the Committee on Food Research of the Quartermaster Food and Container Institute for the Armed Forces. The opinions and conclusions contained in this report are those of the authors. They are not to be construed as necessarily reflecting the views or indorsement of the War Department.

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The use of dehydrated foods has been accompanied in some cases by a reduced acceptability. In some instances this may have been due to an unsatisfactory processing in the preparation of such products; in many more cases, it was probably related to an inadequate training or knowledge of the methods of preparation of such foods by the chefs. Methods which are satisfactory for the preparation of foods in the quantity needed by a small family may be unsatisfactory when used for the preparation of foods in the quantities required by the average army mess. However, processing difficulties have been largely overcome with increasing experience and the products now available are markedly superior to those available during the early days of the war. Greater experience in the preparation of dehydrated foods has lessened the failures encountered in the kitchen.

There has been some question about the satiety value of dehydrated foods as compared with the corresponding fresh foods. If satiety value is related to bulk, and a complete rehydration of such dried foods did not occur in their preparation, such assumptions might have some scientific basis. Other changes might also occur during processing which might affect the acceptability of the dehydrated food.

The present study was undertaken to determine whether dehydrated foods had the same nutritive value as fresh foods when tested by such methods as the determination of their digestibility, their ability to provide nitrogen equilibrium and to maintain body weight. The experiments were also designed to determine how the acceptability of dehydrated foods compares with that of fresh foods. It was hoped that the tests might give some information as to whether any alteration in water balance might accompany the use of such dried foods.

EXPERIMENTAL

Subjects

The studies were carried out on 8 male volunteers who were patients at the Rancho Los Amigos.[4] Some of them were

[4] This is one of the chronic units of the Los Angeles County Hospital System.

the same individuals who had served on a previous study on methionine (Johnson et al., '47). The men ranged from 32 to 56 years of age, from 123 to 179 lbs. in weight and from 66 to 75 inches in height. They were not suffering from any ailments which would be expected to alter their digestive processes or their nitrogen metabolism. The subjects were confined to one ward which was exclusively used for this test. A nurse or attendant was present at all times and during the day 2 nurses and an attendant were on duty. The subjects were allowed to leave the ward but only under supervision. Cigarettes, cigars and newspapers were furnished and the diets were more elaborate than the usual hospital fare. These factors combined with the personal attention made it possible for us to obtain cooperative subjects in spite of the monotony and prolonged confinement necessary for the experiments reported here.

Diets

The diets were prepared in the diet kitchen at the Rancho Los Amigos, and the portions weighed out for the individual trays at the ward. The entire meal was eaten by the subject. The amount of water taken in was also controlled. During the last period of each test, the subjects were allowed to have the food *ad libitum* in additional portions of ¼, ½, or a portion equal to that usually taken with the stipulation that increased amounts of all components of the diet be consumed. In this way an attempt was made to obtain an index of the acceptability of the food.

Four menus were employed each of which provided approximately 12 gm of nitrogen and 2,000 cal. The order in which they were served was in some cases varied to prevent prediction of menu, but during each 8-day metabolic period, each menu was repeated twice. The components of the diets are given in table 1.

The composition of the 4 menus is indicated in table 2. Menu IV was served on Sunday since the cold meal in the evening made it the most practical for that day.

TABLE 1

Diet components used in the studies on fresh and dehydrated foods.

FRESH FOOD DIETS	DEHYDRATED FOOD DIETS
Canned beef, chicken, ham, lamb, pork or veal [1]	
Margarine	
Coffee	
Sugar	
Fresh Idaho potatoes [2]	Dehydrated diced potatoes [2]
Bread	Crackers
Apricots (canned, water-packed)	Dried apricots [3]
Peaches (canned, water-packed)	Dried peaches [3]
Whole milk	Spray-dried whole milk powder [4]
Fresh eggs	Dried egg white [5]
	Dried egg yolk [5]
Orange juice [6]	Orange juice concentrate [7]

[1] Specially prepared by arrangement with Dr. Victor Conquest, Armour and Co.

[2] Obtained from Rogers Brothers, Idaho Falls, Idaho.

[3] Obtained through the courtesy of Dr. Emil Mrak, University of California at Berkeley.

[4] Challenge brand.

[5] Obtained from the Seymour Packing Co., Topeka, Kansas.

[6] Valencia oranges used.

[7] Concentrate from Valencia oranges obtained from the California Fruit Growers Exchange, Ontario, California.

Analytical methods

Nitrogen determinations were made on the urine, dried feces or aliquot of the mixed food sample by the macro Kjeldahl method. Urinary creatinine was determined colorimetrically using the Klett-Summerson colorimeter.

The analyses of the food samples were carried out on an aliquot of combined samples of extra meals weighed out at the regular mealtime. The samples from each day were thoroughly comminuted in a Waring blendor and the contents were made up to approximately 3800 ml with measured amounts of water. This mixture was sufficiently liquid to be pipetted for nitrogen or water determinations. Fat was determined by the Soxhlet method on the dried residue.

TABLE 2

The composition of the different menus employed.

BREAKFAST		DINNER		SUPPER	
Food	Amt.	Food	Amt.	Food	Amt.
	gm		gm		gm
		Menu I			
Scrambled egg	35	Lean roast beef	85	Potato soup	218
with diced ham	10	French fried		Margarine	7
Hashed brown		potatoes	125	Peaches	100
potatoes	205	Margarine	7		
Margarine	14	Apricots	100		
Sugar	35				
		Menu II			
Egg omelet	54	Roast pork	50	Cold sliced beef	50
with diced ham	10	Buttered potatoes	206	Oven browned	
Diced browned		Margarine	14	mashed potatoes	138
potatoes	205	Apricot whip	87	Margarine	14
Margarine	14			Peaches	100
Sugar	35				
		Menu III			
Scrambled eggs	45	Roast veal	40	Mashed potatoes	188
Fried potatoes	177	Baked potato		with diced	
Margarine	14	(no skin)	200	roast lamb	25
Sugar	30	Margarine	14	Margarine	7
		Baked custard	150	Peach in gelatin	153
		Menu IV			
Potato cake	187	Roast chicken		Potato salad	200
Margarine	14	(½ light and		Margarine	9
Sugar	7	½ dark meat)	80	Apricots	100
		Mashed potatoes	188		
		Gravy	65		
		Margarine	9		
		Orange sherbert	166		

Additional foods served.

All meals — Bread 30 gm
Whole milk 169 gm (I and IV); 113 (II and III).

Breakfast — Black coffee, 180 gm
Strained orange juice, 183 gm (I, II, III); 198 gm (IV).

The diets in the 3 series of tests were calculated to contain approximately 12 gm of nitrogen. However, the protein levels were somewhat lower during the period when the dehydrated foods were fed. The variations in nitrogen determined on food samples in the several series are given in table 3.

TABLE 3

Nitrogen content of diets as determined by analysis.

SERIES	MENU I	MENU II	MENU III	MENU IV
Series A (fresh)	12.88	12.99	11.32	13.61
5 samples each	(12.30–14.17)	(12.47–13.19)	(10.04–12.28)	(12.74–14.12)
Series B (dehydrated) [1]	11.36±0.20	10.51±0.37	10.70±0.23	12.88±0.16
8 samples each	(10.83–12.74)	(9.20–12.48)	(9.87–12.20)	(12.14–13.64)
Series C (fresh) [1]	13.28±0.26	12.99±0.17	12.54±0.15	13.69±0.18
10 samples each	(12.20–15.15)	(12.22–13.73)	(11.60–13.00)	(12.74–14.72)

The values in parentheses are the maximum and minimum values.

[1] Includes standard error of the mean calculated as follows $\sqrt{\Sigma d^2/n}/\sqrt{n}$ where "d" is the deviation from the mean and "n" is the number of observations.

Plan of the experiments

The subjects were placed on the diets generally for 2 days as an orientation period before the collections of urine and feces were started. The urine collection was started at 7 A.M. prior to breakfast on each day. No markers were used in the separation of the feces and collections were begun simultaneously with those of the urine. It was considered that the 16- to 32-day periods employed were sufficient to minimize any errors to this procedure.

In the first series of tests carried out from December 3 to December 23, 1946, where fresh foods were employed (series A), only 3 periods were studied. The first 2 were each of 8 days duration when fixed amounts of the foods were ingested, while the third was an *ad libitum* period of 5 days. The second series of tests (B), which were made on dehydrated foods included three 8-day tests on the fixed diets followed by an 8-day *ad libitum* period (January 6–February 7, 1947). The third

series of tests on fresh foods consisted of four 8-day periods followed by an *ad libitum* period of 8 days (February 17–March 28, 1947).

RESULTS

Table 4 records the data on changes in body weight as well as the figures on which protein and water exchanges are based. The results are the averages of 8 subjects for series A and B and for 7 for most of period C. One subject (no. 8) was transferred from the hospital so that he was no longer available for the third series of tests; another subject (no. 4) became ill during period IV of series C. However, since urinary creatinine was normal up to this period and no albumin or sugar appeared, the earlier data are included in the averages.

In spite of the fact that considerable losses in weight occurred during each period, the men were able to recover most of their weight during the *ad libitum* and the rest periods between series A and B and series B and C. The 2000 calorie level sufficed to maintain weight equilibrium with the shorter, lower weight subjects but was quite inadequate for 3 of the men who were tallest and heaviest.

The protein level was sufficiently generous so that the men were in positive nitrogen balance during all periods. The urinary nitrogen was somewhat lower during the periods when dehydrated foods were fed which is probably largely accounted for by the lower protein intake. Fecal nitrogen was quite constant throughout and gave identical averages in the periods with fresh and dehydrated diets. Although considerable fluctuations in urinary creatinine occurred in individual subjects from day to day, the average values for each period are quite constant for each subject. The variations may in part be related to variations in the intake of creatine since the different meats used certainly contained varying quantities of creatine. The average creatinine excretion per day was found to be identical on the fresh and dehydrated diets. We believe the creatinine fluctuations were not due to failure to secure quantitative collection of the urine.

TABLE 4

The average body weight, nitrogen balance, water exchange and urinary creatinine of 8 male subjects who received diets of fresh or dehydrated foods.

CATEGORY OF INTEREST	FRESH FOOD (A)			DEHYDRATED FOOD (B)				FRESH FOOD (C)					AVERAGE	
	I	II	Average	I	II	III	Average	I	II	III	IV	Average	A + C	B
Number of subjects	8	8		8	8	8		7	7	7	6			
Body weight, lbs.[1]														
Start			155.8				153.8					154.0	154.9	153.8
Change[2]			—2.45				—3.05					—3.90	—3.18	—3.05
Nitrogen, gm/day														
Food	12.60	12.75	12.68	11.36	11.01	11.12	11.16	12.83	13.13	13.25	12.65	12.95	12.86	11.16
			±0.25				±0.24					±0.15	±0.13	±0.24
Urine	10.39	10.60	10.50	9.72	9.18	9.74	9.55	11.03	10.25	10.54	9.44	10.34	10.40	9.55
			±0.27				±0.27					±0.36	±0.24	±0.27
Feces	0.84	0.80	0.82	0.92	0.74	0.84	0.84	0.91	0.75	0.95	0.89	0.88	0.85	0.84
			±0.06				±0.05					±0.06	±0.04	±0.05
Balance	1.37	1.35	1.36	0.72	1.09	0.53	0.78	0.88	2.05	1.66	2.32	1.71	1.58	0.78
			±0.26				±0.25					±0.36	±0.25	±0.25
Creatinine, mg/day	1519	1531	1525	1487	1370	1630	1496	1707	1455	1420	1372	1492	1504	1496
			±47				±53					±60	±42	±53
Water, gm/day														
Food	1898	1961	1930	2017	2007	2022	2015	1930	1936	1903	1897	1916	1921	2015
Urine	1346	1351	1349	1272	1196	1349	1273	1331	1302	1292	1352	1313	1329	1273
Feces	40	38	39	45	37	44	42	45	41	53	43	45	43	42
Balance	512	572	542	700	774	629	700	554	593	558	502	558	549	700

[1] Values for 6 subjects only who completed all series of tests.

[2] Value at 20 days for series A (including orientation period) and 21 days for series B and C.

The digestibility of the fat was calculated by the methods usually employed (Langworthy, '23). Correction was made for the metabolic fat according to the procedure of Langworthy and Holmes ('15). The pertinent data are summarized in table 5.

TABLE 5

Summary table of the fat ingested and the coefficient of digestibility on 8 male subjects.

SERIES OF TESTS	FAT IN GM PER 8-DAY PERIOD				COEFFICIENT OF DIGESTIBILITY [2]
	Eaten	Total in feces	Metabolic fat [1]	Feces fat (corrected)	
A. Fresh	(529.2) [3]	22.2	10.8	11.4	98.0 (96.0–99.5)
B. Dehydrated	555.8	18.6	10.5	8.1	98.5 (95.0–100.0)
C. Fresh	529.2	16.2	9.2	7.0	98.7 (96.3–100.0)

[1] Weight of dried feces × 0.0989.

[2] The values in parentheses indicate the range of the results obtained.

[3] Based on the values determined in series C where the same diet was employed.

The average wet and dry weights of the stools are included in table 6, along with the nitrogen analyses. These are presented as evidence that on uniform diets, considerable differences in bulk and dry weight of the stools obtain between different subjects. Moreover, there would appear to be a fair degree of uniformity in the nitrogen content of the stools in any one subject but marked variations in the nitrogen levels of the stools from different individuals.

The experiments where *ad libitum* periods were employed were designed to get objective information as to the acceptability of the fresh and dehydrated foods. These data are summarized in table 7. Because period A lasted only 5 days while B and C were for the standard 8-day interval, the response of the subject is calculated as the percentage additional rations consumed during the period. The previous effect of monotony is balanced since two and four 8-day periods preceded the *ad libitum* periods in series A and C, respec-

TABLE 6

The stool bulk, dried weight and nitrogen content of the various subjects on diets of fresh or dehydrated foods.

SUBJECT	WET WEIGHT GM/8-DAY PERIOD			DRIED WEIGHT GM/8-DAY PERIOD			NITROGEN CONTENT OF DRIED FECES			
	Period A	Period B	Period C	Period A	Period B	Period C	Period A	Period B	Period C	Average
1	321.2	378.2	329.1	87.6	96.5	77.2	7.6, 6.6	6.9, 6.4, 6.8	7.5, 7.2, 7.3, 6.9	7.0
2	605.2	562.4	475.4	139.0	134.6	111.3	7.6, 7.4	6.6, 6.8, 7.4	6.8, 7.1, 7.3, 7.2	7.1
3	596.3	657.1	538.8	116.2	116.7	89.3	5.7, 7.2	7.1, 7.3, 7.6	6.8, 7.3, 7.8, 7.4	7.1
4	221.4	225.2	287.2	80.2	82.6	103.1	4.8, 6.7	5.6, 5.5, 5.7	6.0, 5.8, 6.0, 5.8	5.8
5	370.0	418.9	420.2	86.4	100.6	96.0	5.7, 4.7	5.2, 4.7, 6.3	5.8, 5.7, 5.8, 5.8	5.5
6	379.5	524.2	535.3	79.1	113.1	106.4	6.0, 5.6	6.5, 6.1, 5.1	7.2, 6.7, 6.5, 6.2	6.2
7	565.9	547.2	550.6	136.1	116.9	120.2	7.6, 6.0	7.3, 7.2, 7.5	7.4, 7.7, 8.2, 7.6	7.4
8	282.5	221.0	....	94.2	80.4	....	5.9, 6.0	4.6, 5.2, 5.7	.....	5.5
9	....	....	407.1	....	....	117.0	.....	.....	6.2, 6.3, 6.4, 6.2	6.3
Av. 1–7	437.1	473.3	448.1	103.5	108.7	100.5				

TABLE 7

The extra rations requested and eaten during the ad libitum period while receiving the fresh food diet (A or C) or the dehydrated food diet.

SUBJECT NO.	PERCENTAGE EXTRA RATIONS EATEN			
	A	C	B	Average A + C
1	30	52	50	41
2	15	11	23	13
3 [1]	0	—25	—25	—12.5
4	0	0	4	0
5	40	24	64	32
6	40	37	35	38
7	0	0	0	0
8	20	..	38	20
9 [2]	..	44	..	44
Subjects Average 1, 2, 3, 4, 5, 6, 7, 8			31	21

[1] Subject had gained weight and preferred to eat less. Since an opportunity for a reduced intake was not given for period A, these results are not included in average.

[2] Omitted from the average since no comparison was available on diets with dehydrated foods.

tively, compared with three 8-day periods preceding the *ad libitum* period in series B.

DISCUSSION

The present experiments indicate that food dishes prepared from dehydrated products may have as high a degree of acceptability as those made from fresh foods. This does not entirely agree with the experience of the army where difficulties in some cases occurred when such processed foods were used.

There are 2 possible explanations for the above discrepancies. In the first place the quality of the dehydrated foods now available and which we used must in general be higher than in the early war years as a result of the vast amount of experience in such processing. Moreover, the products we employed were quite fresh and in no case showed any deterioration. Likewise, methods of preparation which proved to be entirely adequate for limited quantities of material in some

cases would be unsatisfactory without modification for the amounts needed for an army mess. In testing out recipes for dehydrated potatoes in the present tests, excellent results were obtained in pilot experiments; however, when the same proportions and methods of preparation were employed for the larger amounts required for the diets, failure resulted. The procedures had to be entirely changed before the products prepared on the larger scale had an edibility which compared favorably with those cooked in the pilot experiments.

One criticism that has been leveled against dehydrated foods is the lack of satiety value. This criticism was not borne out in the present experiments although we have no objective tests to report on it. However, the remarks of the subjects and the answers to questions indicated that the diets prepared from dehydrated foods were equally satisfying to them as those prepared from the fresh foods. Although such data are not quantitative, there is evidence from more recent tests that we might have expected to experience an immediate response from the men had the diet not been satisfactory from the standpoint of satiety. When the potatoes were replaced by bread but the same number of calories administered, the men complained repeatedly over the several weeks that the experiments were characterized by continued hunger (Johnson and Deuel, '47).

The digestibility of the diets shows no differences ascribable to the type of food used. The nitrogen loss in the feces which is generally considered to be largely present in the bacteria was identical in the 2 types of diets, being 0.84 and 0.83 gm, respectively, when the fresh or dehydrated diets were fed.

The digestibility of the fat in the 3 series of tests was found to be especially high. The average in both series was 98% or higher where an average of over 60 to 70 gm of fat was consumed daily. Since the fats were primarily made up of margarine fat, cream and egg yolk fat with minimal amounts of the meat fats, the high digestibility is not unexpected. The values previously reported for the digestibility of egg yolk fat in man are 93.8 (Langworthy and Holmes, '17),

for cream, 96.9 (Langworthy and Holmes, '17) and for margarine fat, 97.2 (Deuel, '46). It is therefore evident that as satisfactory fat utilization obtains with the dehydrated diets as with the fresh ones.

Although no determinations were made of the digestibility of other foodstuffs, the uniformity of the dry weight of the feces in the 3 series of tests would seem to offer evidence of the uniformity of assimilation of the other foodstuffs on diets prepared from dehydrated and fresh foods.

Another indication of the relative nutritive value of the 2 types of diets may be gleaned from the urinary nitrogen levels. The average value of 9.54 gm daily for the samples from the subjects during period B is considerably lower than the average of 10.34 gm per day for the subjects who received the fresh food diet (periods A and C). However, this slightly lower figure in the former case must be the result of the lowered consumption of protein when eating the dehydrated diets. Although the positive nitrogen balance is considerably higher for the subjects receiving the fresh food diet as contrasted with the period when they received the dehydrated diets, it is believed that this is to be attributed largely to the higher level of protein fed.

One of the best indices of nutritive value of a food is its ability to maintain body weight in the adult. Since the food intakes in most cases were too low to allow a caloric equilibrium a gradual decline in weight occurred during the experimental periods. The average loss of weight after 21 days on the dehydrated foods diet (3.05 lbs.) was certainly not greater than that after an equal interval on the fresh food diets (3.18 lbs.).

Although the water balance does not include any figures for the water obtained by the combustion of foods nor does it allow for the loss of water by "insensible perspiration," there is no evidence of an upset in the pathways of excretion of the water. The slightly greater positive water balance in the dehydrated series (700 vs. 548 gm) may be explained on a somewhat higher water intake (94 gm) and the lower excre-

tion in the urine in that series. The lower urine volume might be explained because of the lessened diuresis which resulted from the lower urea excretion.

Stool bulk as well as the dried weight of the feces were approximately the same in all 3 series of tests. Marked variations in the quantities excreted by different subjects were noted. Three subjects (2, 3, 7) consistently excreted between 475 and 600 gm of stools during the 8-day interval while 2 other subjects (4, 8) had a maximum stool weight under 300 gm. Similar variations in dry weight are to be noted. In general, the samples which weighed the least were those containing the lowest percentage of nitrogen while the high values in nitrogen were generally associated with a large production of feces.

It would also appear from the data presented here, that the same subject on a uniform diet excretes a stool with fairly uniform nitrogen content. The maximum value for nitrogen obtained in subjects 4, 5 and 8 was 6.0% while the minimum nitrogen level in subjects 1, 3, 7 and 9 was 6.0%.

One must conclude that by the methods available for evaluation of nutritive value in human subjects, namely digestibility, urinary nitrogen, stool bulk, weight retention and acceptability, dehydrated foods compare satisfactorily with the comparable fresh foods.

SUMMARY

1. Prolonged feeding tests were made on 8 male subjects with fresh food diets and comparable diets made from dehydrated foods.

2. Protein and fat were equally well digested in the diets made from dehydrated and fresh foods. Although the positive nitrogen balance was somewhat higher on the fresh food diets, it is probably due to the somewhat higher level of protein in those tests. Considerably lower urinary nitrogen values were obtained on the diets of the dehydrated foods than on those made from fresh food. Losses in body weight on the 2000 calorie level were similar on the 2 types of diets.

3. The average loss of nitrogen in the feces was identical on the diets of fresh and dehydrated foods.

4. No alterations in the pathway for the metabolism of water were found.

5. The acceptability of the dehydrated food diet as determined by *ad libitum* tests was equally good in the fresh and dehydrated foods.

6. Stool bulk showed wide variations between the different subjects but was relatively constant on any one subject irrespective of the diet.

7. Considerable uniformity was noted in the nitrogen percentage in the dried stools from any one subject on a fairly constant diet although marked variations were noted in the values obtained on the stools of the different subjects.

8. A slightly positive nitrogen retention was observed in subjects receiving the diets prepared from the fresh or dehydrated foods in spite of the fact that they were losing body weight because of low caloric intake.

9. It is concluded that the diets made from dehydrated foods may be equally as nutritious as those prepared from fresh foods.

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THE THIAMINE AND RIBOFLAVIN CONTENT OF WHOLE WHEAT, NONENRICHED AND ENRICHED FLOURS AND OF BREADS MADE THEREFROM

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TWO FIGURES

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The cereals have long been regarded as very important constituents of the human diet, especially that of low-income peoples. In many countries, the process of preparing wheat for human consumption has changed greatly during the past century. With these changes in processing or milling practices, there has arisen some concern as to the probable effects of modern milling methods on the nutritive value of wheat products. Of special concern has been the effect of the milling process, in the production of white flour, in removing certain portions of the wheat berry which are high in essential vitamins and minerals. The nutritional virtues of whole wheat flour and so-called dark breads, as contrasted to those of white flour and white bread, have been proclaimed by numerous adherents. Likewise the superior keeping qualities, the greater public acceptability, more desirable baking properties, and other characteristics of white flour have been similarly propounded. Suggestions have been advanced as to how the nutritive value of white flours may be improved by incor-

[1] Now associated with the Bristol Laboratories, Syracuse, New York.

porating in them, or in the breads made therefrom, certain vitamins, minerals and protein supplements.[2]

Among the more recent publications of experimental work dealing with various comparisons of whole wheat flour and enriched white flour is that of Westerman and Bayfield ('45) who studied the B-complex vitamin content when these products constituted 30 to 50% of the diet of the test animals. These studies included flours enriched in accordance with the previous standard as well as in accordance with the present standard, and it was found that whole wheat was a better source of the B-complex vitamins than was either the "Morris type" flour or the white flour which had been enriched on the basis of the previous standard when these products constituted 30 to 50% of the diet. At the 30% level of feeding, the whole wheat was found to be slightly better than white flour that had been enriched in accordance with the present standard. Whole wheat and enriched flour were found to promote the same rate of growth when fed at the 40% level, while at the 50% level enriched flour promoted a faster rate of growth than did whole wheat.

The authors failed to offer an explanation as to why whole wheat, when fed at the 30% level, supported better growth in rats than did the white flour which had been enriched according to the present formula, while at the 50% level of feeding the enriched flour produced greater gains in body weight than did the whole wheat. From the above report it would appear that either the flour which had been enriched according to the present standard contained dietary essentials not present in the wheat berry or that the natural vitamins (thiamine and riboflavin), although present in the whole wheat in somewhat lower concentration, were utilized more efficiently than were the synthetic vitamins added in the enrichment process, especially when the wheat and the flour

[2] A literature résumé (Monograph) by Dunlap "White versus Brown Flour" was published in 1945. The enrichment of white flour and white bread in the United States began in 1941, and an excellent review may be found in the National Research Council publication, Bulletin no. 10, November, 1944, "Enrichment of Flour and Bread — A History of the Movement."

were incorporated in the diet at or near the critical level (30%).

From reported researches it is difficult to determine the validity of many of the conclusions that have been drawn regarding the relative nutritional merits of whole wheat, white flour and enriched white flour. This is partly due to the fact that the origin of the wheat and of the flours has not been stated in some instances, and where this information has been given it was obvious that frequently the flours under investigation had not been made from the wheat to which their nutritive value was compared.

It appears that the most reliable basis on which to compare the relative nutritive values of whole wheat and wheat flours would be to have all products under investigation come from the same natural source; in this instance, from the same wheat. It also appears that a study of this particular type, especially when wheat and flours of the same origin were involved, would not be complete unless the investigation also included similar studies with breads made from the wheat and the flours in question.

The studies reported at this time were conducted with the view of ascertaining the effectiveness of the present enrichment practice in supplementing the thiamine and the riboflavin content of white flour and also how much of these vitamins is retained by breads made from the wheat and from these flours when the breads are made in accordance with standard baking procedures. These studies have involved only the type of enriched flour produced under the present enrichment formula which has been in effect since October 1, 1943.

EXPERIMENTAL

Materials tested

As basic materials for use in the investigation, 200 lbs. of each, whole wheat, nonenriched flour and enriched flour,

all having a common origin, were obtained.[3] The flour had been produced from the wheat through the usual commercial milling practice and the enriching of the flour had been carried out on a commercial scale. The wheat contained 2.32% nitrogen while the flours contained 2.08% nitrogen (moisture-free basis). The flour was of 62% extraction. The whole wheat, the nonenriched white flour, and the enriched white flour were each thoroughly mixed, transferred to air tight containers and stored at 60°F. until made into bread or until incorporated into the various experimental diets. As it was used, the whole wheat was ground to a high degree of fineness by means of a power driven mill.

Inasmuch as there have been arguments presented in favor of dark bread and of milk bread just as there are arguments in favor of whole wheat bread and of enriched bread, it was decided to include both dark bread and milk bread in the present studies. The dark bread was made from a mixture of flours consisting of 30% whole wheat flour and 70% nonenriched white flour. The milk bread was made by incorporating 1.5 lbs. of dry milk solids (non-fat) in a bread mix containing 25 lbs. of the nonenriched flour. The compositions of the 5 bread mixes are given in table 1. Sufficient distilled water was added to the mixed ingredients to form a heavy dough. The doughs were made into approximately pound loaves and baked in the usual manner for 25 minutes at 450°F. This was carried out in a local bakery operating under commercial conditions.

The baked breads were removed from the oven, taken immediately to the laboratory and weighed. The breads were then crumbled and subjected to preliminary dehydration by being exposed to air at room temperature. The final dehydration was carried out by allowing the breads to remain overnight in a large hot air oven operating at 160–162°F. The dried breads were ground, placed in air-tight containers

[3] The authors wish to acknowledge the cooperation of General Mills, Inc., Minneapolis, Minnesota, and Merck and Co., Inc., Rahway, New Jersey, in making these studies possible.

TABLE 1

Composition of breads.

COMPONENTS	WHOLE WHEAT BREAD	NONENRICHED BREAD	ENRICHED BREAD	DARK BREAD	MILK BREAD
	lbs.	*lbs.*	*lbs.*	*lbs.*	*lbs.*
Flour	25.0	25.0	25.0	25.0	25.0
Sugar	1.0	1.0	1.0	1.0	1.0
Salt	0.5	0.5	0.5	0.5	0.5
Shortening (Crisco)	0.5	0.5	0.5	0.5	0.5
Yeast food	0.1	0.1	0.1	0.1	0.1
Milk powder (skim)	..	..			1.5
Malt syrup	2.0 oz.	2.0 oz.	2.0 oz.	2.0 oz.	2.0 oz.

Water was added to the above ingredients in a sufficient amount to form a heavy dough. The dough was made into approximately pound loaves and baked in the usual manner for 25 minutes at 450°F. This was carried out through the cooperation of a local bakery operating under commercial baking procedure.

and stored at approximately 36°F. until incorporated in the various diets. The changes in the moisture content of the various breads during baking and during subsequent drying are indicated by the data presented in table 2.

Plan of testing

Since the preponderance of evidence indicated that the prewar American diet contained approximately 30% of its calories in the form of cereals, this percentage offered the basis for incorporating the ground whole wheat, the nonenriched flour, the enriched flour and the breads made from these products in the various diets in order to compare their relative thiamine and riboflavin content. Hence the ground whole wheat and the white flours were incorporated in the diets at a level equivalent to 30% of the original flour. This was done by taking into consideration weight changes due to added ingredients other than flour and to moisture additions and losses. The ground wheat, flours and breads replaced an equal weight of sucrose from the basal diet. All diets were made up at frequent intervals and were stored at 36°F. until used. The percentage composition of the 9 basal diets employed as well as the amounts of B-vitamin supplements used are

TABLE 2

Changes in the moisture content of the breads during baking and subsequent drying.

	WHOLE WHEAT BREAD	NONENRICHED BREAD	ENRICHED BREAD	DARK BREAD	MILK BREAD
Amount of flour used, gm	11,360	11,360	11,360	11,360	11,360
Amount of moisture in flour, %	10.52	12.07	11.34	10.95	12.07
Total weight of ingredients, gm	12,305	12,305	12,305	12,305	12,980
Number of loaves produced	40	38	39	40	40
Weight of fresh bread, gm	17,000	15,900	15,925	16,154	16,970
Water uptake, gm	4,695	3,595	3,620	3,949	3,990
Weight of dried bread, gm	11,600	11,180	11,100	11,120	11,825
Residual moisture in dried bread, %	1.85	2.91	1.66	2.45	2.55
Water uptake minus water loss, gm	—705	—1,125	—1,205	—1,085	—1,155
Bread/flour ratio	1.02	0.98	0.98	0.98	1.14

given in table 3. The vitamin supplements other than thiamine, riboflavin and niacin were incorporated directly in the diets, while thiamine, riboflavin and niacin were fed separately. The thiamine, riboflavin and niacin contents of the flours, breads and diets were determined at regular intervals during the course of the feeding tests. In carrying out these assays, thiamine was determined in the enzyme digested extracts by the method of Conner and Straub ('41), riboflavin by the Snell and Strong microbiological method ('39) except

TABLE 3

Percentage composition of the basal diets employed.

DIET NUMBER	1	2	3	4	5	6	7	8	9
Ingredients									
Purified casein	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0
Salt mixture	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0
Hydrogenated cottonseed oil	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0
Cod liver oil	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Cell-U-Flour	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Sucrose	64.0	34.0	34.0	34.0	34.0±	34.0±	34.0±	34.0±	34.0±
Whole wheat flour [2]	...	30.0	...	...	...	...	...	...	...
Nonenriched flour [2]	...	...	30.0	...	...	...	...	...	...
Enriched flour [2]	...	...	...	30.0	...	...	...	...	...
Whole wheat bread [1,3]	...	...	...	...	30.0±	...	...	...	...
Nonenriched bread [1,3]	...	...	...	...	...	30.0±	...	...	...
Enriched bread [1,3]	...	...	...	...	...	...	30.0±	...	...
Dark bread [1,2,3]	...	...	...	...	...	...	...	30.0±	...
Milk solids bread [1,2,3]	...	...	...	...	...	...	...	...	30.0±
Other B-vitamins [4]	+	+	+	+	+	+	+	+	+

[1] All breads were included in the diets on the basis of content of original flour (30% of flour in the diet), the differences in weight between bread and flour being compensated for by adjusting percentage of sucrose.

[2] Made from 30 parts of whole wheat flour and 70 parts nonenriched flour.

[3] Contained 6 parts of skim milk solids.

[4] Supplemented with 200 μg pyridoxine hydrochloride, 800 μg Ca pantothenate, 100 mg choline chloride, 4.0 mg *p*-aminobenzoic acid and 4.0 mg inositol per 100 gm of diet.

[5] The thiamine, riboflavin and niacin contents of these flours and breads (in mg %), in the order listed, were as follows: thiamine — 0.43, 0.06, 0.46, 0.39, 0.07, 0.43, 0.12 and 0.08; riboflavin — 0.09, 0.03, 0.29, 0.08, 0.02, 0.24, 0.04 and 0.14; niacin — 3.52, 0.69, 3.97, 3.58, 0.66, 3.76, 1.39 and 1.09, respectively.

that the concentrations of glucose and of sodium acetate in the finally diluted medium were increased to 2.0 and 2.6%, respectively, as suggested by Stokes and Martin ('43), and niacin by the Krehl, Strong and Elvehjem ('43) modification of the Snell and Wright method ('41).

Diet 1 served as the control since it was essentially free of thiamine, riboflavin and niacin but contained all of the other dietary ingredients known to be required by the growing rat. Diets 2, 3 and 4 contained the flours while diets 5, 6, 7, 8 and 9 contained the breads made from these flours.

TABLE 4

Analysis of diets.

DIET NO.	MOISTURE	ASH	ETHER EXTRACT	CRUDE FIBER	PROTEIN (N × 6.25)	N.F.E.[1]	VITAMIN CONTENT		
							Thiamine	Riboflavin	Niacin
	%	%	%	%	%	%	mg %	mg %	mg %
1	1.46	3.25	9.94	1.25	16.88	67.22	0.00	0.02	0.03
2	4.95	3.74	10.96	2.09	21.56	56.80	.14	.05	1.11
3	4.31	3.33	10.14	1.26	20.93	60.03	.02	.03	.26
4	4.83	3.26	10.59	1.20	21.06	59.06	.14	.12	1.36
5	2.21	3.86	12.51	2.00	21.44	57.98	.11	.04	1.20
6	3.04	3.46	11.17	1.19	21.50	59.64	.02	.02	.19
7	2.53	3.56	11.68	1.28	21.06	59.89	.13	.10	1.16
8	2.19	3.99	11.42	1.84	20.88	59.68	.04	.03	.45
9	2.84	4.71	11.25	1.37	21.25	59.58	.03	.06	.33

[1] Nitrogen-free extract.

The compositions of the 9 diets as well as their average thiamine, riboflavin and niacin content, as revealed by analyses, are given in table 4. Each of the 9 diets was fed unsupplemented, supplemented with riboflavin and niacin, with thiamine and niacin, and with thiamine, riboflavin and niacin. While no beneficial effects were expected as the result of the niacin supplementation, this vitamin was included among the supplements so as to approach conditions comparable to those found in whole wheat flour or in enriched flour. In carrying out the supplementation, the thiamine, riboflavin and niacin were dissolved separately in a 10–90%

alcohol-water solution and were fed daily in the following amounts: 13.2 μg, 8.0 μg, and 105.7 μg, respectively. These dosages were calculated to be the equivalent of the intake of these vitamins when the test animals were subsisting on the enriched flour diet and were consuming, on an average, 10 gm of the diet daily.

Test animals

All test animals employed in the investigation were taken from our breeding colony. A total of 38 experimental groups comprising 450 young rats were used. Thirty-seven of the experimental groups contained 12 animals each (6 males and 6 females), while the remaining group consisted of only 6 animals (3 males and 3 females). The animals of this latter group [group 1 (2d)] received the control diet supplemented by a double portion of the 3 vitamins.

The young rats were taken from the breeding colony when they were 21–23 days of age, at which time their weight ranged between 40 and 45 gm (average 43.3 gm). The animals were placed directly in individual all-metal cages which were provided with raised screen floors (2 meshes per inch) and were subjected to a 2-week adaptation period. During this period each animal received distilled water and a liberal allowance of the control diet (diet 1) without the supplement of thiamine, riboflavin and niacin. At the end of the 2-week adaptation period, the animals were distributed into experimental groups while taking into consideration the usual precautions to distribute litters, sexes and body weights uniformly throughout the various groups. Since the total number of test animals involved was considerable and could not be readily handled at one time owing to other phases of research in progress, the feeding tests were divided into 2 comparable series, and in consequence, only one-half of the animals comprising each experimental group was placed on experiment at one time. At the conclusion of the first series of tests, a second and similar series of feeding tests was conducted.

In all of these tests the diet was fed *ad libitum* and clean distilled water was kept before the animals at all times. The thiamine, riboflavin and niacin supplements were fed in supplement cups independent of the test diet. Daily observations were made for the purpose of detecting early indications of thiamine and riboflavin deficiencies. A careful record was made of individual food consumption and of changes in body weight. All animals remained on experiment for 8 weeks (exclusive of the 2-week adaptation period) unless death intervened.

DATA

The data obtained in the 2 series of feeding tests have been gathered together and reduced to tabular and graphic form and are presented in tables 1 to 4 and in figures 1 and 2.

DISCUSSION

Response of rats receiving the control diet

During the first 7 days of the 14-day adaptation period, the test animals consumed, on an average, 32 gm of the thiamine-riboflavin-niacin free diet (diet 1, table 3) and made favorable growth responses (average of 7.3 gm). However, the response during the last 7 days of the 14-day period was remarkably different. The overall average food consumption was only 18 gm per animal and more than 80% of the rats either failed to gain in weight or lost weight. The average net weight change for the 450 test animals during the 7-day period was a loss in weight of 1.5 gm per rat. While cessation of growth at this time was apparently due to thiamine deficiency, no outward appearance of such a deficiency was evident other than loss of appetite and cessation of growth. At this time the test animals were distributed into the several experimental groups.

Fig. 1 The average food consumption and the average growth response of those groups of rats which received the control diet (diet 1) and the flour-containing diets (diet 2 — whole wheat flour; diet 3 — nonenriched flour; and diet 4 — enriched flour), unsupplemented and supplemented with riboflavin and niacin, with thiamine and niacin, and with thiamine, riboflavin and niacin.

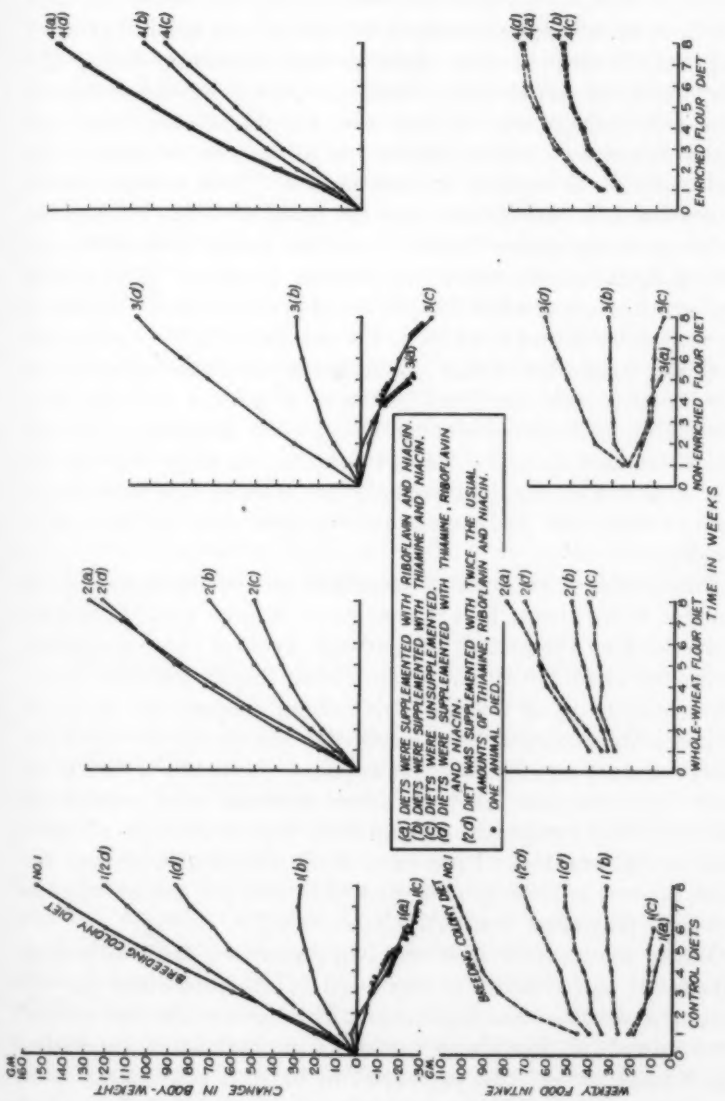
CHANGE IN BODY-WEIGHT
GM.
BREEDING COLONY DIET NO.1
1(a)
1(b)
1(d)
1(2d)
WEEKLY FOOD INTAKE
BREEDING COLONY DIET NO.1
1(a)
1(b)
1(c)
1(d)
1(2d)
CONTROL DIETS
2(a)
2(b)
2(c)
2(d)
3(a)
3(b)
3(c)
3(d)
WHOLE-WHEAT FLOUR DIET
NON-ENRICHED FLOUR DIET
4(a)
4(b)
4(c)
4(d)
ENRICHED FLOUR DIET
TIME IN WEEKS
(a) DIETS WERE SUPPLEMENTED WITH RIBOFLAVIN AND NIACIN.
(b) DIETS WERE SUPPLEMENTED WITH THIAMINE AND NIACIN.
(c) DIETS WERE UNSUPPLEMENTED.
(d) DIETS WERE SUPPLEMENTED WITH THIAMINE, RIBOFLAVIN AND NIACIN.
(2d) DIET WAS SUPPLEMENTED WITH TWICE THE USUAL AMOUNTS OF THIAMINE, RIBOFLAVIN AND NIACIN.
• ONE ANIMAL DIED.

Figure 1

Those animals which were continued on the basal diet and which received a supplement of riboflavin and niacin [group 1 (a), fig. 1] made a very slight growth response during the first week of supplement feeding. However, the response was only temporary in that the animals constituting the group again declined in weight and all succumbed before the end of the sixth week of the test period. These results served to confirm our expectation that the basal diet was reasonably deficient in thiamine. When a similar group of animals received the thiamine-niacin supplement [group 1 (b)], a slow but consistent growth response resulted which continued throughout the first 6 weeks of the test period. This indicated that the basal diet either contained measurable amounts of riboflavin or that the body stores of riboflavin had not been completely eliminated during the 2-week adaptation period. Since the test animals did not continue to grow during the last 2 weeks of the test period, the authors are inclined to believe that the prolonged growth was due to the latter condition.

The group of rats which remained on the unsupplemented control diet [group 1 (c)] continued to lose weight, and all were dead by the end of the seventh week of the test period. However, since the animals comprising this group lived somewhat longer, on an average, than did a comparable group of animals subsisting on the same diet but receiving the riboflavin-niacin supplement, it appears that the feeding of additional amounts of 1 or more vitamins may accentuate the deficiency symptoms arising from lack of another vitamin. Similar observations have been made frequently during the past several years in connection with other types of vitamin studies (Guerrant et al., '37).

When the control diet was supplemented with thiamine, riboflavin and niacin [group 1 (d)], an immediate growth response resulted which continued throughout the test period. The animals of this group gained an average of 86 gm during the 8-week period and appeared to be in a reasonably good state of nutrition at the completion of the feeding test. Since

Westerman and Bayfield ('45) had found that flour enriched in accordance with the present formula, when fed at the 50% level, did not produce optimal growth in young rats, the question remained as to whether the suboptimal growth was due to further deficiencies of thiamine and riboflavin or to other characteristics of the experimental diet. In consequence, the second group of young rats (only 6 animals) receiving the control diet was given a double allowance of the thiamine-riboflavin-niacin supplement. While these animals [group 1 (2d)] made increased growth responses (15 gm per week) as the result of receiving the additional allowance of vitamins, they did not attain the growth rate of young rats subsisting on our breeding colony diet (group 1) during the same period (19.3 gm per week). Whether further supplementing of the diet with thiamine and riboflavin would have brought about additional improvements in the growth rate cannot be stated inasmuch as additional tests were not made. However, the data obtained are sufficient to show that the primary deficiencies in the control diet used in the present studies, insofar as limiting growth in young rats, were those of thiamine and riboflavin.

Responses obtained with the flour-containing diets (diets 2, 3 and 4 of table 3)

When 30% of freshly ground wheat was incorporated in the control diet in place of a similar weight of sucrose (diet 2), definite improvement in the growth rate resulted [diet 2 (c), fig. 1]. All animals comprising the experimental group responded immediately to the change in diet and grew continually during the 8-week test period. While the growth rate was not optimal (average of 6.4 gm per week) the results demonstrated that the ground wheat used in these studies, when incorporated in the ration at the 30% level, furnished sufficient thiamine and riboflavin to sustain life and to support a slow but consistent rate of growth. When this latter diet was supplemented with riboflavin and niacin [group 2 (a)] good growth resulted (129 gm in 8 weeks) and the animals

appeared to be in excellent condition at the end of the test period. Although the thiamine-niacin supplement proved less effective in promoting growth in young rats [group 2 (b)] subsisting on this diet than did the riboflavin-niacin supplement, the animals did respond to the former supplement and grew at an average rate of 9.0 gm per week in comparison with 6.4 gm gains made by comparable animals receiving the unsupplemented diet. This would suggest that 30% of ground whole wheat, when used to replace an equal weight of sucrose from the control diet, did not furnish adequate thiamine for optimal growth and that additional growth could be brought about by feeding a thiamine supplement. However, when the whole wheat flour diet was supplemented with thiamine, riboflavin and niacin [group 2 (d)] no greater increases in body weight were obtained than those observed without the thiamine supplement. Under the conditions of these experiments the feeding of the thiamine-riboflavin-niacin supplements did result in a slightly improved growth rate during the first 5 weeks of the test period, but this advantage disappeared during the next few weeks. While this latter observation remains unexplained, the data obtained in this phase of the investigation confirm previous reported observations that riboflavin deficiency is of primary consideration where whole wheat and wheat products are concerned.

Growth studies with diets containing 30% of nonenriched flour, unsupplemented and supplemented with riboflavin and niacin, with thiamine and niacin, and with thiamine, riboflavin and niacin [groups 3 (a, b, c and d)] yielded results which proved to be remarkably similar to those obtained under comparable conditions while using the control diet. However, there was evidence that the diet carried somewhat more thiamine and definitely more riboflavin as the result of including the 30% of nonenriched flour. Here again the rats receiving the riboflavin supplement, in the absence of thiamine, died at an earlier date than did comparable animals which did not receive the riboflavin.

When 30% of enriched flour replaced an equal weight of sucrose from the control diet, satisfactory growth responses were obtained both with and without additional vitamin supplements [groups 4 (a, b, c and d)]. In fact more favorable growth was obtained in all 4 instances than was obtained under comparable conditions of experimentation in which the diet contained 30% of ground whole wheat. Here again the thiamine supplement proved less effective in promoting additional growth than did riboflavin. In regard to the data presented in figure 1, it is of interest to note the consistent relationship between food intake and growth.

Responses obtained with bread-containing diets

Growth studies with diets containing the breads in amounts equivalent to 30% ground wheat or 30% of the flours from which the breads were made (diets 5, 6, 7, 8 and 9, table 3 and fig. 2) yielded data which were remarkably similar to those obtained with the previously described flour-containing diets (fig. 1). In most instances, growth was slightly greater on the bread diets than on the corresponding flour-containing diets. Mortality was also lower among the thiamine-deficient bread-fed animals. These apparent differences in nutritive value of the flour and of the bread-containing diets may, perhaps, be attributed to 2 possible factors, namely, to the more readily accepted bread diets and to the probable addition of vitamins or other growth stimulating nutrients in connection with the making of the breads. In regard to the former possibility, it can be definitely stated that in 9 of the 12 comparisons, the bread-fed animals consumed on an average more of the diet than did the flour-fed animals, while in 2 comparisons the amount of diets consumed by the 2 groups of animals was the same, and in one comparison the bread-fed rats consumed slightly less food than did the flour-fed animals. As to the latter possibility, it is recognized that traces of thiamine and riboflavin may have been added to the bread-containing diets by incorporating small amounts of yeast food,

malt syrup and live yeast culture in the bread mix (table 1). However, periodic analyses of breads and diets (tables 3 and 4) failed to show that the breads contained any more thiamine or riboflavin than did the flours from which they were made. In fact the analytical data indicate that the breads had sustained a slight loss in these vitamins apparently as the result of baking, drying and other preparatory measures preliminary to feeding.

Studies with the whole wheat bread diet [groups 5 (a, b, c and d)] reveal that sufficient thiamine was present to support satisfactory growth and that only a slight improvement in growth resulted when additional thiamine was fed. On the other hand, when the diet was supplemented with riboflavin, with and without additional thiamine, an increased rate of growth resulted, just as it had with the corresponding flour-containing diet. Although the animals which received a diet containing the bread made from the nonenriched flour [groups 6 (a, b, c and d)] grew at a slightly faster rate than did those animals receiving the flour diet, the responses obtained through feeding of the 2 diets, unsupplemented and supplemented, were remarkably similar.

The groups of rats receiving the diet containing the bread made from the enriched flour, without and with vitamin supplementation [groups 7 (a, b, c and d)], grew at approximately the same rate as did the animals receiving the corresponding flour diet. Here again the data clearly show that the amount of riboflavin added through enrichment was not adequate for optimal growth when the enriched flour or the bread made thereform was incorporated in the diet at the 30% level, although the amount of this vitamin present in the enriched flour was somewhat greater than that found in the whole wheat or the whole wheat bread.

Fig. 2 The average food consumption and the average growth response of groups of rats which received the bread-containing diets (diet 5 — whole wheat bread; diet 6 — nonenriched bread; diet 7 — enriched bread; diet 8 — dark bread; and diet 9 — milk bread), unsupplemented and supplemented with riboflavin and niacin, with thiamine and niacin, and with thiamine, riboflavin and niacin.

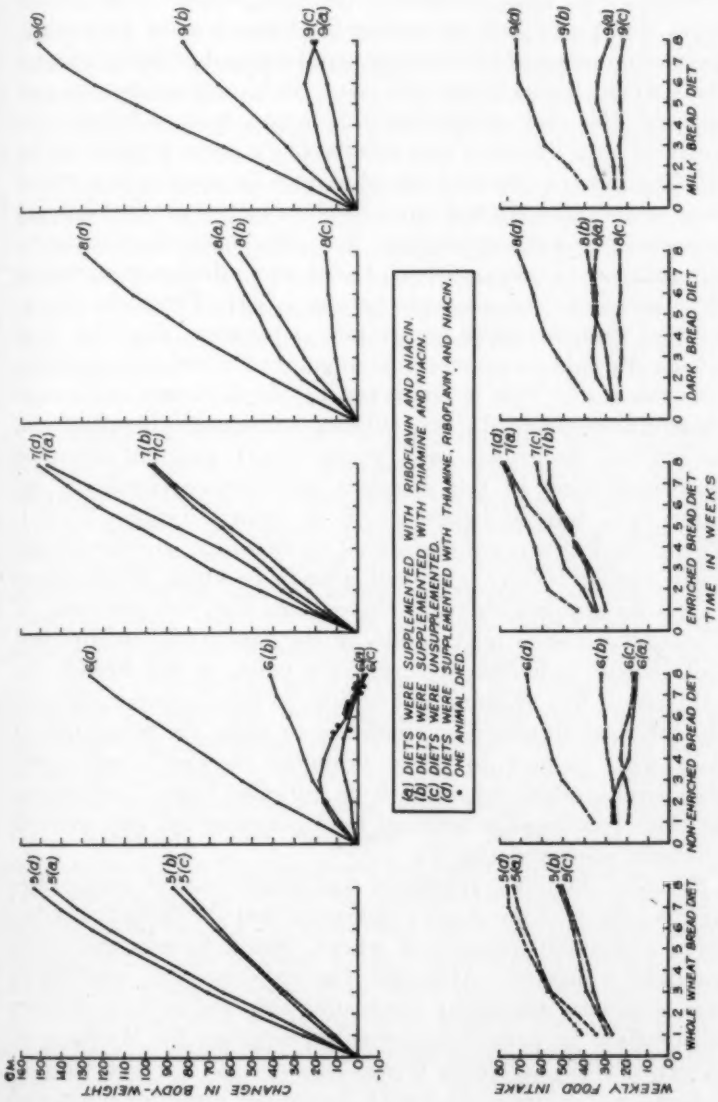
CHANGE IN BODY-WEIGHT
WEEKLY FOOD INTAKE
WHOLE WHEAT BREAD DIET
NON-ENRICHED BREAD DIET
ENRICHED BREAD DIET
DARK BREAD DIET
MILK BREAD DIET
TIME IN WEEKS
(a) DIETS WERE SUPPLEMENTED WITH RIBOFLAVIN AND NIACIN.
(b) DIETS WERE SUPPLEMENTED WITH THIAMINE AND NIACIN.
(c) DIETS WERE UNSUPPLEMENTED.
(d) DIETS WERE SUPPLEMENTED WITH THIAMINE, RIBOFLAVIN, AND NIACIN.
• ONE ANIMAL DIED.

Figure 2

Studies with diets containing the dark bread (30% ground whole wheat and 70% of nonenriched flour) show that while the incorporation of a relatively small amount of finely ground whole wheat in the bread did result in improved growth and survival, the diet containing this bread was definitely deficient in both thiamine and riboflavin [groups 8 (a, b, c and d)]. The incorporation of 6% of skim milk powder in a bread made of the nonenriched flour resulted in the product having increased growth-stimulating and life-sustaining capacity [group 9 (c) vs. group 6 (c)], but it was inferior to enriched white bread in these respects [group 7 (c)]. From the standpoint of vitamin supplementation, it appears that the milk powder did add an appreciable amount of riboflavin but very little thiamine. This is borne out by the fact that additional riboflavin supplementation, without additional thiamine, did not improve the growth rate [group 9 (a)], and that thiamine supplementation in the presence of existing riboflavin resulted in a marked improvement in growth [group 9 (b)]. When this diet was supplemented by thiamine, riboflavin and niacin [group 9 (d)], excellent growth resulted. While these results do support existing contentions that the addition of skim milk or skim milk solids, in the making of unenriched white bread, enhances the nutritive value of the bread, the inclusion of 6% of skim milk solids, in this instance, did not add sufficient thiamine or riboflavin to make the bread equivalent with respect to these vitamins to the bread made from ground whole wheat or from enriched flour. Here again the close relationship between food consumption and growth is readily observed (fig. 2).

Periodic thiamine, riboflavin and niacin assays made during the course of the studies indicated that the thiamine, riboflavin and niacin content of wheat, flours, breads and diets remained constant. Although the experimental conditions were somewhat dissimilar, the results obtained in these studies substantiate in part those recently reported by Westerman and Hall ('47) in which it was shown that further supplementation of enriched flour with B-complex vitamins improved

the nutritive value of the product. In the present studies this was found to be true of riboflavin supplementation. Furthermore, riboflavin supplementation of the whole wheat diets was more effective in stimulating additional growth in young rats than when this vitamin was employed as a supplement to the enriched flour diet.

While the present investigation has been concerned primarily with the relative thiamine and riboflavin content of whole wheat, nonenriched flour and enriched flour, the authors are aware that whole wheat may contain desirable nutrients other than thiamine and riboflavin in greater amounts than are present in either the nonenriched flour or the enriched flour.

SUMMARY

A study was made of the relative thiamine and riboflavin content of whole wheat, nonenriched and enriched flour of common origin and of breads made therefrom. Four hundred and fifty young rats constituting 38 experimental groups were used in the study. All animals were subjected to a 2-week adaptation period before being placed on experiment. The feeding period was of 8 weeks duration unless death of experimental subject intervened. The results, as a whole, show that flour, enriched in accordance with the present formula, is definitely superior to nonenriched flour with respect to thiamine and riboflavin, and is somewhat superior to whole wheat with respect to these vitamins. While the amount of thiamine contributed by ground whole wheat and by enriched flour, when the flours composed 30% of the diet, is only slightly less than that required for optimal growth in young rats, the amount of riboflavin contributed is definitely insufficient. However, the enriched flour was found to contain more riboflavin than the original wheat. Breads made from ground wheat and from enriched flour show good retention of thiamine and riboflavin as indicated by growth tests and by vitamin assays. Dark bread and milk bread, as prepared and used in these studies, were found to be definitely inferior to breads made from ground whole wheat or from enriched flour

as sources of thiamine, and somewhat less inferior as sources of riboflavin.

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EDITORIAL REVIEW

EVALUATION OF AMINO ACID REQUIREMENTS BY OBSERVATIONS ON THE CHICK[1]

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ONE FIGURE

(Received for publication July 25, 1947)

Investigations of amino acid requirements of chicks conducted by the author and others in the Division of Poultry Husbandry of the University of California over a period of several years, have given rise to a mass of data of a very homogenous nature. Most of the data available have met or approached closely to the following specifications: (a) uniformity in breed and strain of chicks used, (b) pre-test rearing period of 10 to 14 days with an excellent practical diet, (c) experimental period of 10 days, (d) protein or equivalent amino acid levels at 20% or more of the diet, (e) diets substantially complete except for the amino acid under investigation.

The objective of this review was, in part, to establish amino acid intake levels to be recommended by the sub-committee on Poultry Nutrition of the National Research Council. During the course of the analysis of these data there have come to light several relationships of quite fundamental interest.

Two principal variables will be considered throughout this discussion, namely, (a) the percentage of the amino acid in the diet, as determined by additions of the crystalline amino

[1] Presented at the eleventh Annual Meeting, American Institute of Nutrition, Chicago, 1947.

acid or by the content of the amino acid in the protein component, and (b) the percentage daily change in body weight of the chicks, which will be referred to simply as rate of gain. Grau ('47a) has studied such factors as forced feeding, body composition, *ad libitum* feeding and body weight changes of chicks. He has come to the conclusion that change in body weight on an *ad libitum feeding* program is the most satisfactory criterion of dietary adequacy in amino acid studies with chicks. Similarly, Hegsted and Worcester ('47) have found with rats that proteins are classified relative to each other with high accuracy by gain in weight alone.

Where it has been shown that D— or DL— forms of the amino acid are efficiently utilized, these forms have been considered equivalent to the L— form. All protein content data are based on percentage N $\times$ 6.25. The manner of evaluating data is illustrated for certain amino acids in tables. Curves relating amino acid content in the diet to rate of gain are presented in figure 1. In the construction of these curves individual points have been given due weight on the basis of numbers of chicks used in the experiment. Data have not been used for one amino acid when it is apparent that another amino acid deficiency is also exerting an effect in the same region of growth rate. Because of limitations of space, not all of the figures and tables originally prepared can be presented here. A sufficient number of each are given to illustrate the findings in this study.

Arginine

The data for arginine given in table 1 and plotted in figure 1 fall substantially on a straight line over the range of 0.17% to 1.2% arginine, in diets containing whole proteins. It formerly appeared that this requirement was approximately 1% of the diet (Klose et al., '38). However, the upper arginine curve of figure 1 in the region of maximal rate of gain, which is at least 7%, indicates that the optimal arginine content of the diet may be nearer to 1.2%. The question has been raised of the degree of availability of arginine in casein (see Klose

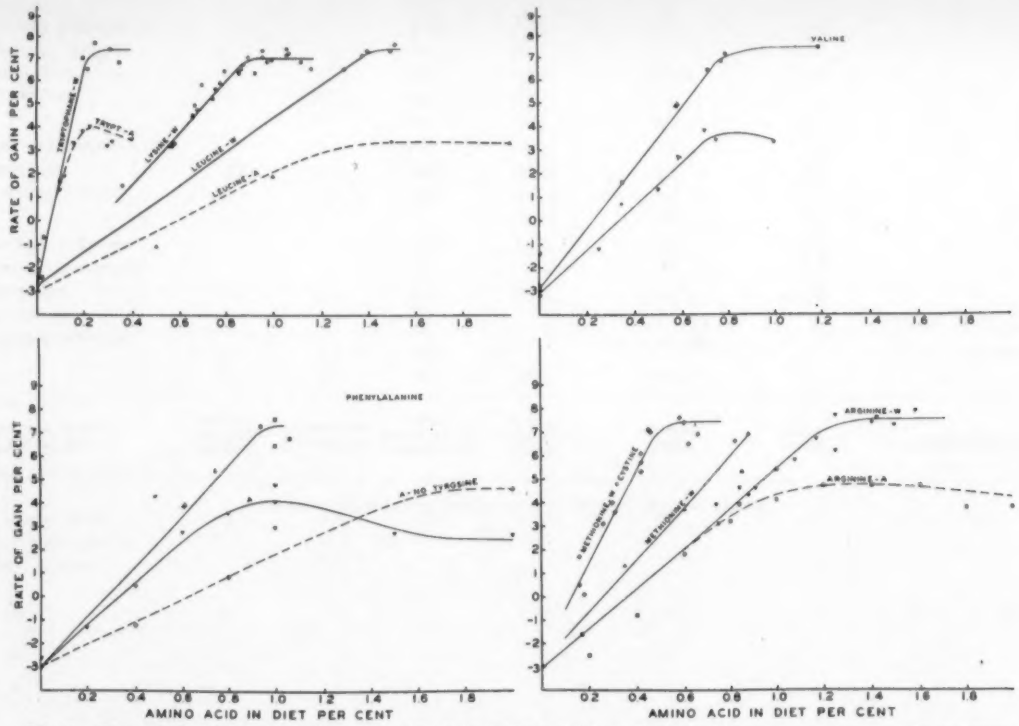

Fig. 1 The relation between daily rate of gain in body weight of chicks to the percentage of certain indispensable amino acids in the diet. Curves marked "W" refer to whole proteins diets . . . those marked "A" to diets containing amino acids or hydrolysed proteins in place of whole proteins.

TABLE 1

Relation of arginine in the diet to rate of gain of chicks.

AMINO ACID SOURCES IN DIET	PROTEIN SUPPLIED TO DIET	L-ARGININE IN PROTEIN	L-ARGININE SUPPLIED TO DIET	TOTAL L-ARGININE IN DIET	RATES OF GAIN	AVE. RATE	REFERENCES
	%	%	%	%	%	%	
Hydrolysed arginine-free casein	18	0.0	0.0		—1.5,—1.5 —1.5,—1.8		Klose et al. ('38)
Polished rice	2.4	7.2	0.17	0.17	—1.6,—1.9	—1.6	
Cystine, tryptophane Above plus arginine			0.83	1.00	4.1	4.1	
Hydrolysed casein	18	3.7	0.67				
Polished rice	2.4	7.2	0.17	0.84	3.9	3.9	
Cystine, tryptophane							
Whole casein	18	3.7	0.67				
Polished rice	2.4	7.2	0.17	0.84	4.5,4.8	4.6	
Cystine Above plus arginine			0.41	1.25	7.3,8.1	7.7	
Whole casein	27	3.7	1.00				
Polished rice	2.4	7.2	0.17	1.17	6.3,7.0	6.7	
Above plus arginine			0.17	0.91	4.6	4.6	
			0.34	1.08	5.8	5.8	
			0.51	1.25	6.2	6.2	

Whole casein	27	3.7	1.00				
Polished rice	2.4	7.2	0.17	1.17	6.3,7.0	6.7	
Above plus arginine			0.17	0.91	4.6	4.6	
			0.34	1.08	5.8	5.8	
			0.51	1.25	6.2	6.2	
			0.75	1.49	7.3	7.3	
Amino acid mixture	20	0.0	0.0	0.0	—3.0	—3.0	
Above plus arginine			0.2	0.2	—2.5	—2.5	
			0.4	0.4	—0.8	—0.8	
			0.6	0.6	1.8	1.8	
			0.8	0.8	3.2	3.2	
			1.0	1.0	5.4	5.4	
			1.2	1.2	4.7	4.7	
			1.4	1.4	4.7	4.7	
			1.6	1.6	4.7	4.7	
			1.8	1.8	3.8	3.8	
			2.0	2.0	3.8	3.8	
			2.2	2.2	4.5	4.5	
Casein	18	3.7	0.67				Klose and Almquist
Yeast	2	4.0	0.08	0.75	2.9,3.2	3.1	('40)
Glycine, cystine							
Soybean meal	20	7.1	1.42	1.42	7.6,7.9	7.6	Almquist et al.
Cystine, methionine					7.4		('42)
Corn gluten meal	20	4.4	0.88	0.88	4.3	4.3	Grau ('46)
Lysine, tryptophane							
Cystine, glycine							
Fish meal	20	7.0	1.40	1.40	7.4	7.4	Almquist ('46)

et al., '38), and if it should be proven that this availability is not complete, the optimal arginine requirement will be somewhat lower than indicated in this report. The lower arginine curve based upon data from amino acids or diets with hydrolysed-protein reaches a maximal gain at approximately the same percentage of arginine in the diet. Both arginine curves extend to the vicinity of a —3% rate at complete deficiency; this is consistent with similar curves for the other indispensable amino acids.

Methionine

It is well-known that the methionine requirement cannot be considered without giving attention to the cystine intake, since the latter is not only synthesized at the expense of the former, but also has a valuable "sparing action" on methionine.

The available data for methionine have been classified in 2 ways, namely, (a) with more than the maximal cystine requirement present, and (b) with substantially no cystine present (table 2). The corresponding curves are also given in figure 1. The curves are practically straight lines up to the region of optimal gain. Both methionine curves extrapolate into the zone of negative gains to an intercept close to —3%.

In the presence of an adequate cystine level in the diet the methionine requirement for optimal gain is close to 0.5%; while the requirement in the relative absence of cystine is close to 0.9% of the diet. These estimates remain in agreement with previously suggested requirements (Almquist, '45).

Lysine

The data on lysine are remarkably consistent. The curve (fig. 1) attains an upper limit for growth at close to 0.9% lysine in the diet (Almquist and Mecchi, '42). All of the data are from diets based upon whole proteins, but these are of a considerable variety, as will be seen from table 3. The linear portion of the curve (fig. 1) extrapolated to zero lysine would reach the vicinity of —3%.

TABLE 2

Relation of methionine in the diet to rate of gain of chicks. Adequate cystine and choline present.

AMINO ACID SOURCES IN DIET	PROTEIN SUPPLIED TO DIET	METHIONINE IN PROTEIN	L- OR DL-METHIONINE SUPPLIED TO DIET	TOTAL L- OR DL-METHIONINE IN DIET	RATES OF GAIN	AVERAGE RATE	REFERENCE
	%	%	%	%	%	%	
Arachin	18	0.5	0.09				
Gelatin	5	0.6	0.03				Klose and Almquist ('41)
Yeast	3	2.0	0.06				
Alfalfa	0.2	2.3	0.004	0.18	0.1	0.1	
Cystine							
Arachin	23	0.5	0.12				
Gelatin	7	0.6	0.04	0.16	1.7	1.7	Almquist and Jukes ('42)
Lysine, threonine							
Cystine, tryptophane							
Valine, tyrosine							
Same as above				0.16	0.5	0.5	Almquist et al. ('43)
Beef blood cells	20	1.4	0.28				Grau and Almquist ('44a)
Gelatin	5	0.6	0.03	0.31	3.6	3.6	
Cystine, isoleucine							
Isolated soybean protein	20	1.5	0.30	0.30	3.6,3.9,4.1,4.2	4.0	Almquist and Grau ('45)
Cystine					4.3		
Above plus methionine			0.12	0.42	5.3	5.3	
Soybean meal	20	2.1	0.42				Almquist et al. ('42)
Gelatin	5	0.6	0.03	0.45	7.1	7.1	
Cystine, tryptophane							
Above plus methionine			0.13	0.58	7.6	7.6	

TABLE 2 *(continued)*

AMINO ACID SOURCES IN DIET	PROTEIN SUPPLIED TO DIET	METHIONINE IN PROTEIN	L- OR DL-METHIONINE SUPPLIED TO DIET	TOTAL L- OR DL-METHIONINE IN DIET	RATES OF GAIN	AVERAGE RATE	REFERENCE
	%	%	%	%	%	%	
Corn gluten meal	20	2.1	0.42	0.42	5.9,6.2	6.1	Grau ('46)
Arginine, lysine							
Cystine, tryptophane							
Glycine, valine							
Cottonseed meal	20	2.1	0.42	0.42	5.7,5.7	5.7	
Lysine							
Above plus methionine			0.20	0.62	6.9,6.3,6.2	6.5	
			0.40	0.82	6.6	6.6	
Peanut meal, lysine	20	1.3	0.26	0.26	3.1	3.1	
Above plus methionine			0.20	0.46	7.0	7.0	
			0.40	0.66	6.9,6.4,7.5	6.9	
Fish meal	20	3.0	0.60	0.60	7.4	7.4	Almquist ('46)
Substantially no cystine,[1] adequate choline present.							
Isolated soybean protein	20	1.5	0.30	0.35 [1]	1.1	1.1	Grau and Almquist ('43)
Above plus methionine			0.25	0.60	3.7,3.7,3.8	3.7	
			0.50	0.85	5.3	5.3	
Casein	20	3.6	0.72				Almquist and Mecchi ('41)
Gelatin	10	0.6	0.06	0.89 [1]	6.7,7.0,6.8,7.0	6.8	

[1] Traces of cystine in the diets have been added to the methionine.

TABLE 3

Relation of lysine in the diet to the rate of gain of chicks.

AMINO ACID SOURCES IN DIET	PROTEIN SUPPLIED TO DIET	LYSINE IN PROTEIN	L-LYSINE SUPPLIED TO DIET	TOTAL L-LYSINE IN DIET	RATES OF GAIN	AVERAGE RATE	REFERENCE
	%	%	%	%	%	%	
Zein	30	0.2	0.06	0.06	—1.7	—1.7	Almquist and Mecchi ('42)
Arginine, methionine, threonine, tryptophane, glycine			0.40	0.40	0.2	0.2	
Above plus lysine			0.80	0.80	6.4	6.4	
Edestin	25	2.3	0.58	0.58	3.3	3.3	
Glycine, methionine							
Above plus lysine			0.10	0.68	4.7	4.7	
			0.20	0.78	5.9	5.9	
			0.30	0.88	6.7	6.7	
			0.40	0.98	6.8	6.8	
Edestin	25	2.3	0.58				
Casein	5	8.5	0.42	1.00	6.9	6.9	
Sesame meal	20	2.8	0.56	0.56	3.2	3.2	Grau et al. ('46)
Above plus lysine			0.10	0.66	4.4	4.4	
			0.20	0.76	5.6	5.6	
			0.30	0.86	6.3	6.3	
			0.40	0.96	7.3	7.3	
Arachin	23	1.5	0.34				
Gelatin	7	4.4	0.31				Almquist et al. ('43)
Lysine			0.10	0.75	5.2	5.2	
Methionine, cystine, tryptophane, valine							

TABLE 3 (*continued*)

AMINO ACID SOURCES IN DIET	PROTEIN SUPPLIED TO DIET	LYSINE IN PROTEIN	L-LYSINE SUPPLIED TO DIET	TOTAL L-LYSINE IN DIET	RATES OF GAIN	AVERAGE RATE	REFERENCE
	%	%	%	%	%	%	
Arachin	18	1.5	0.27				Klose and Almquist ('41)
Gelatin	5	4.4	0.22				
Yeast	3	6.8	0.20				
Alfalfa	0.2	5.0	0.01	0.70	5.8	5.8	
Tryptophane, threonine, methionine							
Sesame meal	20	2.8	0.56	0.56	3.2	3.2	Grau and Almquist ('44c)
Above plus lysine			0.50	1.06	7.4	7.4	
Soybean meal	10	5.8	0.58				Almquist and Grau ('44a)
Sesame meal	10	2.8	0.28	0.86	6.4	6.4	
Soybean meal	13.3	5.8	0.77				
Sesame meal	6.7	2.8	0.19	0.96	7.0	7.0	
Corn gluten meal	20	1.8	0.36	0.36	1.5	1.5	Grau ('46)
Arginine, cystine, tryptophane, glycine							
Cottonseed meal	20	3.3	0.66	0.66	4.4,4.3	4.4	
Above plus lysine			0.37	0.93	6.2,6.3	6.3	
			0.46	1.12	6.6,6.9	6.8	
Peanut meal	20	3.0	0.60	0.60	5.5	5.5	
Methionine, threonine							
Tryptophane							
Above plus lysine			0.46	1.06	6.9,7.0,7.5	7.1	
Water-extracted linseed meal	20.3	3.3	0.67	0.67	4.9	4.9	Kratzer et al. ('47)
Above plus lysine			0.20	0.87	6.5	6.5	
			0.40	1.07	7.2	7.2	

Tryptophane

The tryptophane data (table 4) are based upon diets with whole proteins, hydrolysed proteins and amino acids. The curve for whole proteins (fig. 1) extends from an optimal gain at 0.25% *l*-tryptophane (Almquist, '45) down toward the same limiting value common to the other amino acids. The data from amino acid or hydrolysed-protein diets form a separate curve at the higher region of gain, which, however, agrees with the other curve as to the point at which further additions of tryptophane do not cause further increases in growth rate.

Leucine

The leucine curve at 0% leucine is definitely located by experimentally determined rates of —2.7 to —3.0% (fig. 1) (Almquist and Grau, '44b). At the upper end the curve is located only by the minimum contents of leucine known to be present in several whole protein sources which, with necessary supplements, have been shown to support good gains. Hence, the leucine requirement for optimal gain does not exceed 1.4% of the diet. The data from amino acid diets (Grau and Peterson, '46), although lower in magnitude of growth, pass through the same maximum region at 1.4% leucine.

Phenylalanine.

Like methionine, phenylalanine may be spared by another amino acid, in this case tyrosine. The data (Almquist and Grau, '44b; Grau, '47a) have been separated on the basis of adequate tyrosine or no tyrosine present in the basal diets. This procedure establishes 2 curves. The first, for adequate tyrosine present, goes through a maximum at close to 0.9%; while the second curve, for no tyrosine present, attains a maximum at approximately 1.6% of the diet. The ends of both of these curves at 0% phenylalanine reach a determined growth rate near —3%.

A probable curve for whole protein diets has been indicated also. The lower end of this curve should obviously be near

TABLE 4

Relation of tryptophane in the diet to the rate of gain of chicks.

AMINO ACID SOURCES IN DIET	PROTEIN SUPPLIED TO DIET	TRYPTOPHANE IN PROTEIN	L-TRYPTOPHANE SUPPLIED TO DIET	TOTAL L-TRYPTOPHANE IN DIET	RATES OF GAIN	AVERAGE RATE	REFERENCE
	%	%	%	%	%	%	
Hydrolysed casein	20	0.0	0.0				
Gelatin	10	0.1	0.01	0.01	—2.4,—2.2	—2.3	Almquist and Mecchi ('41)
Above plus tryptophane			0.15	0.16	3.3	3.3	
Hydrolysed casein	18	0.0	0.0				Klose et al. ('38)
Polished rice	2.4	1.3	0.03		—0.7	—0.7	
Cystine							
Above plus tryptophane and arginine			0.20	0.23	3.9,4.1	4.0	
Casein	18	1.2	0.22				
Polished rice	2.4	1.3	0.03	0.25	7.3,8.1	7.7	
Cystine, arginine							
Casein	27	1.2	0.32				
Polished rice	2.4	1.3	0.03	0.35	6.3,6.5,7.0,7.2	6.8	
Cystine							
Corn gluten meal	20	0.5	0.10	0.10	1.7	1.7	Grau ('46)
Arginine, lysine, cystine, glycine							
Peanut meal	20	1.0	0.20	0.20	7.0	7.0	
Lysine, methionine, threonine							
Cottonseed meal	20	1.1	0.22	0.22	6.9,6.3,6.2	6.5	
Lysine, methionine							
Hydrolysed casein	15	0.0	0.0				Grau and Almquist ('43)
Gelatin	10	0.1	0.01	0.01	—2.3	—2.3	
Above plus tryptophane			0.25	0.26	3.8	3.8	
			0.50	0.51	3.5	3.5	
Amino acid mixture	20	0.0	0.10	0.10	1.3,1.2,1.2	1.2	Grau and Almquist ('44b)
Above plus L- or			0.20	0.20	3.8,3.8,3.3	3.6	
DL- tryptophane			0.30	0.30	2.9,3.5	3.2	
Zein			0.40	0.40	3.8,3.2	3.5	
Lysine, methionine	20	0.1	0.02	0.02	—2.7	—2.7	
Valine, threonine							
Cystine, gelatin							
Fish meal	20	1.3	0.26	0.26	7.4	7.4	Almquist ('46)

— 3%. The upper end is fixed, by the virtue of growth rates attained with whole proteins of comparatively low phenylalanine content, at not more than 0.9%.

Threonine

Data on threonine requirement are few (Almquist and Grau, '44b; Grau, '47b). At 0% threonine the curve is definitely anchored in the region of — 2 to — 3%. The points are, for the most part, based upon very small groups of chicks. However, the general characteristics of the curves resemble those obtained with other indispensable amino acids. A similar linear relation has, therefore, been assumed. The optimal threonine content in amino acids diets is apparently close to 0.6%. Certain proteins of comparatively low reported threonine content were found capable of supporting optimal chick growth when providing a dietary threonine level of no more than 0.6%. Hence it seems probable that the estimated requirement is correct in both kinds of diets.

Valine

The amino acids diets (Grau and Peterson, '46) attain a maximal growth-promoting value at approximately 0.8% valine. Whole proteins of comparatively low valine content will support optimal gains when furnishing not more than 0.8% valine to the diet. Hence the 0.8% value may be provisionally accepted as the requirement. The lower ends of the curves are experimentally located close to — 3% (Almquist and Grau, '44b; Grau and Peterson, '46).

Isoleucine

The above story is repeated in the case of isoleucine, with the exception that the curve for amino acids diets seems to be displaced slightly toward the left. It is probable, however, that the natural leucine used in the basal diets for this work (Grau and Peterson, '46) may have contained small amounts of isoleucine; as little as 5% would have caused the displace-

ment noted. It is suggested, provisionally, that the isoleucine requirement is close to 0.6% of the diet.

Histidine

The data for histidine are so scarce that no curves could be constructed. On a histidine-free amino acid diet a few chicks lost weight at approximately —2%. A supplement of 0.15% L-histidine may not have been entirely adequate for maximal growth on this diet (Almquist and Grau, '44b). The upper limit of histidine requirement is fixed at not more than 0.30% of the diet. This figure is based on the reported analyses of sources of proteins which have been shown to support optimal growth.

The significance of negative gains

Although protein synthesis in the organism is completely prevented by the lack of an indispensable unit, the processes of protein degradation are not simultaneously stopped. Whether the animal will exhibit loss or gain of protein will depend upon the balance between protein synthesis and protein destruction, both of which are proceeding rapidly, according to modern theory. It is evident from the figures that the complete absence of a truly indispensable amino acid from the diet of the chick results in a loss of weight at a rate in the zone of —2 to —3%. With sufficiently accurate data on all the amino acids in question, it is probable that this zone would have been narrowed toward some value closer to —3. In a few cases where the limiting value is not available from direct experiment, it has been assumed to equal —3% for the purposes of constructing the curves. This assumption is adequately justified from the nature of the data.

The magnitude of the loss of weight is, of course, determined by many factors, such as age and condition of the animal, duration of the deficiency and general experimental procedure, all of which have been comparable in the body of data reviewed. There is nothing fundamental about the pre-

cise numerical value of the limiting negative rate, but its evident constancy under the experimental conditions can only be regarded as of real fundamental significance.

It is obvious that a complete deficiency of any one indispensable amino acid will prevent any protein synthesis in the animal just as absolutely as a complete deficiency of protein in the diet. As a matter of fact, a protein-free diet causes approximately the same rate of weight loss (Grau, '47a). Hence, all these various deficiencies must approach a common limit in effect as the degree of interference with protein synthesis approaches completeness.

In experiments on an entirely different problem (Berg and Rohse, '47) young rats fed gelatin, casein, hydrolysed casein and protein-free diets showed equivalent rates of loss in weight when subjected to a complete deficiency of an indispensable amino acid, or of protein. Rate of growth of rats on a tryptophane-supplemented acid-hydrolysed casein diet was distinctly lower than that on a whole casein diet. These results with rats agree with the general features of the chick results reviewed in the present paper.

It is evident that the point from which to measure growth (as affected by protein synthesis) is not the usually accepted point of zero growth, but some value akin to the —3% limit of the present data. Any rate significantly more positive than the negative limiting value, even though still negative in sign, is evidence that some protein synthesis is taking place. If such rate is obtained with a complete deficiency of a certain amino acid, then that amino acid is also being synthesized.

Amino acid requirements as determined by whole proteins and by amino acid mixture diets

It is noteworthy that in substantially every example in which the maximal growth effect from a particular amino acid has been ascertained with whole-protein diets, as compared to amino acid or hydrolysed-protein diets, the requirement values appear to be in close agreement.

Notwithstanding the fact that the best gains obtained with either hydrolysed proteins or amino acid mixtures are less than the optimal gains to be obtained with whole proteins, it is evident that, in either case, the same relative proportions of amino acids to each other and to the whole diet must be established to secure the maximal gain possible on the particular diet. It is further indicated that quantitative amino acid requirement values should be equally valid whether established with amino acid mixtures or with whole proteins.

It appears from the linear nature of the curves and the agreement of the whole proteins and amino acids diets maxima that the requirement of any indispensable amino acid for any rate of growth has a fixed proportion to the others in the diet. This is compatible with the general concept that protein synthesized by an animal is always the same characteristic set of proteins, hence requires the same proportions of materials to be used in the synthesis. The above findings extend this concept to include any rate of synthesis.

These observations further imply that the proportions of the amino acids reaching the synthetic regions in the animal are determined by the proportions in the diet, if not identical with them, since these proportions are the same whether the amino acids come from a variety of whole proteins or are present in the uncombined state, as in amino acids or hydrolysed proteins.

Growth rate differences observed with complete amino acid mixtures and supplemented hydrolysed proteins as compared to whole proteins

These differences are a reflection of several possible causes, operating singly or in combination.

a. Decreased appetite and feed consumption. Possibly, the more rapid absorption of free amino acids induces a quicker physiological reaction to curtail appetite. This is only a small effect when the amino acid supply is complete, since

then the feed intake is controlled largely by caloric requirements. The marked and almost immediate reduction of feed consumption during a severe amino acid deficiency is perhaps only an extreme stage of the same effect. In such case, the animal cannot as readily dispose of absorbed amino acids by combining them into protein, and is even more quickly satiated. But when the lacking indispensable amino acid is restored to the diet, even though such addition could hardly affect the sensory acceptability of the diet, the increase in the appetite of the animal is very evident.

TABLE 5

The effect of periodical access to feed on the growth of chicks fed diets containing whole protein as compared to the hydrolysed protein. (20% protein in all diets. No other sources of protein in diets.)

SOURCE OF PROTEIN	ACCESS TO FEED	FEED EATEN PER DAY PER CHICK	AVERAGE WEIGHT GAIN PER DAY	GAIN-FEED RATIO
		gm	%	
Fish protein	*ad libitum*	22.9	4.0	.40
Fish protein	2 hrs. A.M. and 2 hrs. P.M.	21.4	4.0	.42
Hydrolysed fish protein	*ad libitum*	20.9	3.5	.34
Hydrolysed fish protein	2 hrs. A.M. and 2 hrs. P.M.	16.0	2.4	.26

In an attempt to demonstrate the effect of appetite curtailment by amino acid mixtures in the diet, an experiment was conducted with chicks fed a diet containing fish protein or a hydrolysate of this protein as the exclusive source of amino acids in the diets. Preliminary observations indicated that an appetite-curtailing effect of hydrolysed protein might be to some extent counteracted by a tendency of the chicken to eat more frequently, if less at any one time. Consequently, groups allowed only periodical access to feed were added to the program. The results are given in table 5.

These results show that the chicks having periodical access to the whole-protein diet were able to consume almost as much

feed and to grow as well as the *ad libitum* fed group. The *ad libitum* group on the hydrolysed-protein diet was able to consume only slightly less feed than the above-mentioned groups, but grew less efficiently on the diet — this is the usual result in such comparisons (Klose et al., '38; Stokstad, '40) and is probably a demonstration of causes listed below. However, the periodical-access group fed hydrolysed protein would not ingest sufficient feed to enable this group to keep pace with the others. This difference is most probably a demonstration of a rapidly effective appetite-curtailing action of the hydrolysed protein. Very similar results were obtained in the same experiment performed with young rats.

b. Increased metabolic destruction of the more rapidly absorbed free amino acids. These may enter the blood stream faster than they can be diverted into protein synthesis, and the surplus undergoes a relatively greater degree of catabolism than would be the case with amino acids whose release depends upon slow enzymatic digestion of protein.

c. Increased loss of the rapidly absorbed free amino acids in the urine.

d. Increased destruction of amino acids by intestinal bacteria.

e. The *release during* the *digestion* of whole proteins of some *simple peptides* which may be *used directly* by the animal in the synthesis of its own characteristic proteins, thus, perhaps, promoting faster protein synthesis than would be the case following ingestion of free amino acids.

f. The presence of a "growth factor" in whole animal proteins, and its absence from amino acid mixtures or completely hydrolysed proteins (Wooley, '45). In regard to the application of such a "factor" to the results of the present study it can only be said that the animals showed continuous, linear growth curves over the time intervals employed, and at no time any indications of increasing depletion in any growth factor. Furthermore, it was found that the decrease in growth-promoting value of a protein upon hydrolysis (corrected for any amino acid losses) was related most directly to the degree

of hydrolysis, and there was no extra value to be found from leaving a few per cent of whole protein in the diet (animal protein factor). There are numerous examples in which the animals showed optimal gains without animal proteins in the diet.

Amino acid requirements to support any rate of growth below the optimal

These may presumably be taken by a horizontal projection across the curves. Thus, for example, the theoretical "maintenance" requirements of the young chick fed a diet containing whole proteins only would be obtained as the intersection of the zero gain level with each of the appropriate curves. In each case the "maintenance" requirement is approximately 30% of the optimal requirement, for the chicks employed in the studies reviewed. This has been experimentally confirmed by feeding diets so low in protein that only the maintenance requirement of several indispensable amino acids was provided.

The curves for the amino acids requirements could be expressed empirically as follows:

$$R = K_1 P - K_2$$

Where R = rate of change in body weight

K_1 = rate constant for change in body weight due to protein synthesis = approximately 10

P = fraction of the optimal level of the amino acid in the diet.

K_2 = rate constant for change in body weight due to protein degradation = approximately -3.

It is evident that meeting only half the requirement for a particular indispensable amino acid will not permit half the optimal rate of growth, but only 2/7 of this optimal rate.

SUMMARY

1. The level of an indispensable amino acid in the diet bears a linear relation to the rate of growth of the chick, from rapid loss of weight at complete deficiency to maximal growth at complete adequacy of the amino acid.

2. All the curves converge toward a common negative rate of gain at complete deficiencies of any indispensable amino acids, since protein synthesis must stop in all such cases.

3. Net gain of the chick depends upon the balance between protein synthesis and protein catabolism, over the entire range of the curves.

4. While chicks grow better on diets in which the amino acids are supplied chiefly in whole proteins, as compared to diets compounded with amino acids or hydrolysed proteins, the maximal requirements determined with these 2 kinds of diets are in good agreement. This is compatible with the concept that on either kind of diet the chick must synthesize the same body proteins, hence requires the same proportions of amino acids for this purpose.

5. These proportions of indispensable amino acids to each other remain the same for any sub-optimal rate of growth, even in the negative zone of growth.

6. Reasons for the differences in the growth supported by whole proteins as compared to hydrolysed proteins and amino acids are discussed.

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PRESERVATION OF CAROTENE IN DEHYDRATED VEGETABLES[1]

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Recently we showed (Halverson and Hart, '47) that carotene could be completely preserved in dehydrated alfalfa and cereal grasses if the material had a water content of 12–15% and was sealed in air-tight receptacles. The phenomenon of preservation involves primarily the utilization of the oxygen with production of carbon dioxide — an atmosphere in which carotene is stable. Respiratory enzymes in the dehydrated material are accelerated in action by raising the water content above that usually contained in dehydrated vegetable materials. If the water content of the material is less than 12%, say 10% or less, then the rate of oxygen utilization and carbon dioxide production is reduced with a variable and not always predictable carotene loss.

To test the application of this method of carotene preservation to dehydrated vegetables we used carrots, spinach (leaf) and broccoli. These materials were purchased fresh, dried at 50°C. and then ground to a fine powder. After drying and grinding the water content was as follows: carrots 10.25%, broccoli 8.00%, and spinach 4.5%. The materials were then placed in pint size cardboard boxes with or without water

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adjustment to higher levels and with or without flexo-wax sealing. The sealing was done to prevent loss of carbon dioxide and access of oxygen — a condition absolutely necessary for carotene preservation, and one which we have found effective in the case of dehydrated alfalfa or cereal grasses. Any receptacle that is air-tight such as glass, metal, cellophane, fabricated paper or cardboard dipped in a non-cracking wax should prove effective.

After preparing the materials they were stored at room temperatures of 22–25°C. for 3 months. At the end of that time carotene determinations were made. The chromatographic method of Wilkes ('46) was used. The results are given in table 1.

The original carotene content of the dehydrated materials on a water-free basis in μg per gm was as follows: carrots 510, broccoli 74.4 and spinach 266.

As expected from our experiments with dehydrated alfalfa and cereal grasses, the carotene loss in 3 months was high in the carrots and broccoli without sealing but not so high in the case of spinach under the same conditions of storage and with the low water content of 4.5%. Under sealed conditions the carotene loss in all the materials decreased in general with increase in water content. With 12.5% of water the losses in all the materials were negligible.

It is apparent from the data that in these dehydrated products the carotene loss can be greatly reduced with sealed conditions and a total water content of 10–12.5%.

In table 1 are also brought together observations on the color, taste, and internal gas pressure as it may have developed. With dehydrated alfalfa and cereal grasses sealed, and with the water content up to 20% no internal gas pressure, involving bulging of the carton storage receptacles, has been observed. This same situation was observed with spinach, but in the case of carrots and broccoli some internal gas pressure developed particularly at the higher water levels. Also darkening of the product, especially in the case of carrots, was observed. To reduce the possibility of gas pressure, as in the

TABLE 1

Observations on character of dehydrated products and carotene losses in 3 months' storage at 22–25°C.

TREATMENT	COLOR	TASTE	AROMA	GAS PRESSURE	CAROTENE CONTENT, μG/GM WATER FREE BASIS			
					% water	Initial	3 months	% loss
			Carrots — dehydrated					
No seal	Reddish brown	Sweet	Pleasant	Negative	10.2	510	150	71.0
Sealed	Reddish brown	Sweet	Pleasant	Negative	10.2	510	361	29.0
Sealed	Deep brown	Bland	Pleasant	Positive	12.5	510	442	13.0
Sealed	Deep brown	Bland	Coffee-like	Positive	15.0	510	469	8.0
			Broccoli — dehydrated					
No seal	Green color	Bland	Pleasant	Negative	8.0	74.4	36.5	51.0
Sealed	Green color	Bland	Strong broccoli	Negative	8.0	74.4	70.0	6.0
Sealed	Olive green	Bland	Strong broccoli	Positive	12.5	74.4	73.2	1.6
Sealed	Olive green	Bland	Strong broccoli	Positive	15.0	74.4	72.6	2.0
			Spinach — dehydrated					
No seal	Bright green	Bland	Pleasant	Negative	4.5	266	215	19.0
Sealed	Bright green	Bland	Pleasant	Negative	4.5	266	236	11.0
Sealed	Bright green	Bland	Pleasant	Negative	10.0	266	260	2.0
Sealed	Olive green	Bland	Pleasant	Negative	12.5	266	261	1.8
Sealed	Olive green	Bland	Pleasant	Negative	15.0	266	252	5.2

case of carrots and broccoli with water levels of 12.5%, the water level could be held at 8–10%. Under such conditions complete carotene preservation may not be obtained, but the loss of carotene under sealed conditions would be greatly reduced. Apparently the application of the principle of a proper water content with sealed storage can be made use of for carotene preservation in dehydrated fresh vegetable tissues. In the case of spinach a water content of 10% plus sealing reduces the carotene loss to practically zero. Whether this observation would be general for spinach is unknown. Usually with other dehydrated plant tissues such as alfalfa, rye, oats and carrots a moisture content of 10% or less does not give a maximum carotene retention observed with moisture levels of 12–15%.

The color of the stored products deepened somewhat with increased water content. However, at 10–12% of total water the color was not greatly different from that of the original product. With 15% of total water the color of the carrots became a deep brown and in the other 2 products an olive green. Broadly speaking, a water content of 10–12% and sealing will not only preserve well the carotene but also give a product resembling the original unsealed material in color, taste and aroma. It is probable that the moisture levels required for maximum carotene preservation will vary with the different vegetables and the method of dehydration employed.

SUMMARY

1. The carotene content of dehydrated spinach, broccoli and carrots was well preserved under storage at room temperature for 3 months by sealing in cardboard receptacles and adjusting the total water content to 10–12%. In the case of carrots and broccoli with the total water content of 12.5% some internal gas pressure developed. With 8–10% of total water no internal gas pressure developed but the carotene loss was greater than at the higher water level.

2. Under sealed conditions of storage with a total water content of 8–10% the taste, aroma and color of the original product were well retained.

3. The principle of preservation involved is mainly the acceleration of the respiratory activity of enzymes present in the dried plant tissue with the result that the oxygen present is replaced with carbon dioxide.

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INTERRELATION BETWEEN α-TOCOPHEROL AND PROTEIN METABOLISM

II. THE INCREASED UTILIZATION OF CASEIN PRODUCED BY α-TOCOPHEROL, YEAST DIGEST, OR XANTHINE IN THE RAT-GROWTH PROTEIN-QUALITY TEST [1]

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ONE FIGURE

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In a previous paper (Hove, '46) it was shown that d, α-tocopherol reduced weight loss in adult rats maintained on a 5% crude-casein diet for extended periods of time. It was pointed out that this result was in apparent contradiction to the work of Dam ('44) in which was noted no influence of α-tocopherol on the body weight of rats maintained until death on a diet containing approximately 5% protein from yeast. It occurred to us that the 4-week, rat-growth method originated by Osborne, Mendel and Ferry ('19) for determining protein quality might be useful in resolving these divergent results.

EXPERIMENTAL

Litter-mate pairs of male albino rats were placed on the test diets at weaning. One member of each pair received daily, 1 mg of d, α-tocopherol in 2 drops (50 mg) of olive oil, while the other received only the olive oil. Body weight and food consumption records were made weekly for the second to fifth

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week from weaning. The protein-efficiency ratios were obtained by dividing the 4-week body-weight gain by the total 4-week protein intake. The composition of the weight gains was not determined. Therefore the effect of tocopherol to be described may have resulted from increased deposition of fat or other constituents, rather than increased protein utilization. However, the custom that has grown up around the use of this method will be followed and results will be expressed as protein efficiency rather than more correctly as food efficiency.

TABLE 1

Composition of diets used.

BASAL MIXTURE		%
	PROTEIN SOURCE SUCROSE	86
	SALT MIXTURE, USP XII, NO. 2 [1]	4
	LARD	10
		per gm of diet
Vitamin supplements	Thiamine-HCl	10 μg
	Riboflavin	10 μg
	Pyridoxine HCl	10 μg
	Calcium pantothenate	25 μg
	Nicotinic acid	50 μg
	2-Methyl, 1-4 naphthoquinone	5 μg
	Choline chloride	1.0 mg
	i-Inositol	0.1 mg
	Vitamin A	50 units
	Vitamin D (Delsterol)	3 units

[1] With added $ZnCO_3$, 0.1%; $CuSO_4 \cdot 7\ H_2O$, 0.08%; and $MnSO_4$, 0.01%.

Ad libitum feeding was allowed in most instances. Diets were weighed into a "Franke" earthenware cup. This cup was contained in a straight-sided aluminum dish. Using this system food spillage was reduced to less than 3 gm weekly for nearly all rats. The spillage was determined by placing collecting papers under the cages and weighing the discard weekly.

The composition of the diets is indicated in table 1. The B-vitamins [2] and vitamin K were thoroughly ground with a small quantity of purified casein or sucrose, as the case might

[2] Merck.

be. Vitamins A and D were added to the lard. Additions of folic acid and biotin have not been made to the diets. The diets were kept refrigerated at all times.

The casein used was a "vitamin-test" product [3] and was assumed for our purpose to be 100% protein. A yeast-protein concentrate [4] prepared by enzymatic digestion was used; this contained 67% protein. The washed wheat gluten contained 70% protein.

In the first experiment casein was used at 5 and 10% levels both with or without the addition of 0.5% *l*-cystine to the diet. The yeast protein was used to the extent of 10 and 15% in the diet, and wheat gluten protein at 15% in the diet. The results are shown in table 2. On the 10% casein diets the α-tocopherol-supplemented rats showed protein efficiency ratios significantly greater than those of the controls. On none of the other diets was there a benefit due to tocopherol.

Some of the groups from this experiment were continued on their diets for 12 weeks at which time the tooth pigment of the maxillary incisors was rated on a 0 to 4 scale. The rats were autopsied and examined for stomach ulcers. The results given in table 3 are similar to those reported previously for casein (Hove, '46). Yeast protein apparently can replace tocopherol as a protective agent against depigmentation.

Furthermore, it will be noted from table 3 that tocopherol supplementation failed to induce an enhanced weight gain when yeast supplied the protein of the diet. Dam's report ('44) in which tocopherol failed to influence body weight and the opposite finding by Hove ('46) that tocopherol had a very significant effect on body weight may now be reconciled. Dam used yeast protein and Hove used casein.

A second experiment was designed to repeat the tocopherol effect on the 10% casein diet and to determine the influence of small amounts of yeast protein on the utilization of casein.

[3] General Biochemicals, Inc.

[4] "Basamin-Busch" from Anheuser-Busch, Inc., St. Louis, Mo.

TABLE 2

Effect of d, α-tocopherol on the utilization of protein by growing rats. Six litter-pairs used per diet. Values are the 4-week averages from the second through the fifth week after weaning.

PROTEIN IN DIET	α-TOCOPHEROL FED DAILY	AVERAGE GROWTH	AVERAGE FOOD CONSUMED	PROTEIN EFFICIENCY RATIO (GROWTH/PROTEIN INTAKE)	
	mg	*gm*	*gm*	± S.E.[1]	$t = \frac{D}{E_d}$[2]
Casein, 5%	0	10.3	156	1.31 ± .115	.034
	1	9.2	145	1.26 ± .094	
Casein, 5% + 0.5% l-cystine	0	15.5	157	1.76 ± .148	0.39
	1	15.5	171	1.68 ± .142	
Casein, 10%	0	39.7	226	1.73 ± .095	2.26
	1	51.3	259	1.99 ± .066	
Casein, 10% + 0.5% l-cystine	0	87.5	301	2.72 ± .084	2.09
	1	96.8	311	2.96 ± .079	
Yeast protein, 10%	0	38.0	240	1.58 ± .095	0.57
	1	38.3	228	1.67 ± .124	
Yeast protein, 15%	0	86.2	320	1.77 ± .103	0.57
	1	88.9	313	1.86 ± .120	
Wheat gluten protein, 15%	0	22.1	170	0.87 ± .031	2.24
	1	19.7	183	0.73 ± .054	

[1] S. E. $= \sqrt{\frac{\text{dev.}^2}{N(N-1)}}$.

[2] t for P = .05 is 2.23. Values greater than this are significant.

TABLE 3

Growth, stomach ulcers, and color of maxillary incisor teeth of rats after 12 weeks on various protein diets.

PROTEIN	DAILY α-TOCOPHEROL	AVERAGE WEIGHT GAIN (12 WEEKS)	STOMACH ULCER INCIDENCE	AVERAGE TOOTH COLOR[1]
	mg	*gm*	%	
Casein, 5%	0	15	66	0.4
	1	18	17	2.6
Casein, 10%	0	117	0	0.5
	1	156	0	2.9
Yeast protein, 10%	0	121	0	1.1
	1	126	0	3.1
Wheat gluten protein, 15%	0	64	33	2.5
	1	60	17	3.3

[1] A score of 0 = a tooth completely bleached; chalk white.
A score of 4 = a tooth normally colored; brownish yellow.

The results of this experiment are shown in tables 4 and 5. With the larger number of animals the influence of α-tocopherol on utilization of protein on the 10% casein diet was more striking. Statistically the difference due to tocopherol is significant with a probability of more than 99 chances in 100

TABLE 4

Effect of 1 mg d, α-tocopherol daily on the utilization of casein at a 10% level in the diet of young male rats. Values are 4-week averages.

EXPERIMENT NUMBER	NUMBER OF LITTER-MATE PAIRS	WEIGHT GAIN (GM)		PROTEIN INTAKE (GM)		PROTEIN EFFICIENCY RATIO (GAIN/PROTEIN)		
		−E[1]	+E[2]	−E	+E	−E	+E	$t = D/E_d$[3]
1	6	39.7	51.3	22.6	25.9	1.73	1.99	(3.25)
2	15	50.5	59.2	22.6	23.2	2.23	2.48	(4.27)
3	5	45.2	45.6	24.1	20.3	1.88	2.23	(3.64)
4	5	35.6	46.2	19.3	22.1	1.85	2.09	(3.73)
5	5	38.8	43.2	21.4	20.7	1.81	2.09	(3.74)
	Average	42.0	48.9	22.0	22.4	1.88	2.18	

[1] Without tocopherol.

[2] With tocopherol.

[3] Student's method for $t = D/E_d$ in which values of t for P = .02 are 3.37, 2.62 and 3.75 for 5, 14 and 4 degrees of freedom, respectively.

TABLE 5

Effect of yeast-protein digest and xanthine on the utilization of casein for growth by young male rats over a 4-week period.

EXPERIMENT NUMBER	PROTEIN SOURCE	NUMBER OF RATS	DAILY α-TOCOPHEROL	PROTEIN EFFICIENCY RATIO (GAIN/PROTEIN) ± S.E.	$t = D/E_d$[1]
			mg		
2	Casein, 10%	15	0	2.23 ± .064	(3.67)
	Casein, 8% + 2% yeast protein	10	0	2.56 ± .063	
	Casein, 8% + 2% yeast protein	10	1	2.50 ± .117	
3	Casein, 10%	5	0	1.88 ± .023	(3.14)
	Casein, 10% + 0.1% xanthine	5	0	2.11 ± .070	
	Casein, 10% + 0.1% xanthine	5	1	2.12 ± .074	

[1] Values for $t = D/E_d$ by the Fisher method in which values of t for P = .01 are 2.81 and 3.36 for 23 and 8 degrees of freedom, respectively.

(table 4). However, the addition of a small amount of yeast protein also increased the utilization of casein and completely replaced α-tocopherol in this action (table 5). The absolute values in this experiment are considerably higher than usual and we have no explanation for this.

Schwarz ('44) has noted that xanthine can replace α-tocopherol in preventing liver damage and delaying death in rats on an alkali-washed casein diet. Therefore, a third experiment was designed to determine the effect of xanthine on casein utilization. From the results given in table 5 it is evident that xanthine does increase the protein efficiency ratio and replaces α-tocopherol in producing this effect. Perhaps α-tocopherol acts at some point in the metabolic changes from dietary amino acids to xanthine. Consequently, in the absence of tocopherol, xanthine becomes a dietary essential.

The effect of d, α-tocopherol on the utilization of casein at the 10% level in the diet has been confirmed in 2 other brief experiments, the results of which are tabulated in table 4.

In the preceding experiments *ad libitum* feeding has been allowed, using diets containing the single level of 10% protein, for the most part. To furnish a clearer insight into the nature of the tocopherol effect another experiment was set up which used an equalized-feeding technique with diets containing a wide range of casein levels.

Diets with purified casein contents of 6, 8, 10, 12, 14, 18 and 40% were prepared. Five litter-pairs of weanling male albino rats were placed on each diet, and tocopherol supplements given as before. Each rat received exactly 7.0 gm of diet daily. Except for 4 rats on the 6% casein diet all daily allotments were consumed. On the 6% casein diet the total refusal was 48 gm for the — E group and 10 gm for the + E group over the 4 weeks of the experiment. Protein efficiency ratios were calculated in the usual way by dividing the 4-week total gain in body weight by the protein contained in the quantity of diet consumed by each rat during this 4-week period. The results are expressed graphically in figure 1. On the 8 and 10% casein diets the increased utilization due to tocopherol

was statistically significant, the respective $\frac{D}{E_d}$ values for those groups being 2.96 and 3.55 (Fisher method). No other differences were statistically significant.

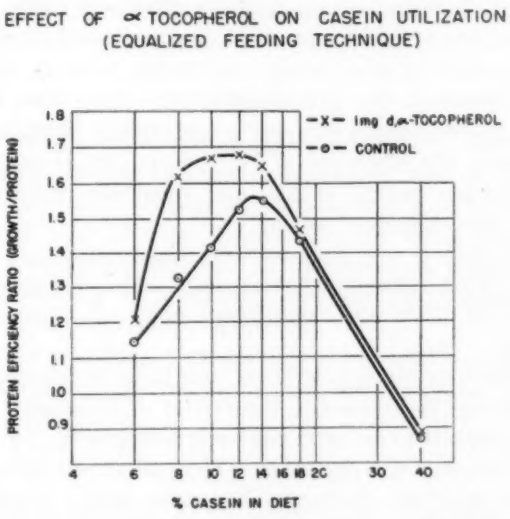

Fig. 1 The influence of d, α-tocopherol on the protein efficiency ratio as determined on diets with varying casein contents. Each point represents the average of 5 rats over 4 weeks. An equalized feeding technique was used in which all animals received 7.0 gm of diet daily.

DISCUSSION

Under the experimental conditions outlined, α-tocopherol produced a small but significant increase in the efficiency of food utilization by growing rats on a diet containing 10% purified casein. The improvement due to tocopherol was statistically significant in each of 5 consecutive experiments employing a total of 72 rats fed *ad libitum*. The average protein-efficiency ratio for the vitamin E-low groups was 1.88, as compared with 2.18 for the tocopherol supplemented groups. The beneficial effect of α-tocopherol was as evident on equalized feeding as on *ad libitum* feeding techniques.

This effect of tocopherol in improving efficiency of food utilization has been noticed by Patrick and Morgan ('43) in chickens fed a synthetic diet containing casein protein. An explanation for the beneficial influence of tocopherol on food utilization efficiency or on protein-efficiency ratio may be found in an experiment described by Bosshardt et al. ('46a). As non-protein calorie intake increased, protein intake remaining constant, the protein-efficiency ratio was found to increase. Consequently, in our experiments tocopherol may be increasing the efficiency of fat metabolism thus giving, in effect, a higher calorie intake and resulting indirectly in a higher protein-efficiency ratio.

Low levels of a yeast protein autolysate, or 0.1% of xanthine added to the low-casein diet, each improved the protein-efficiency ratio and completely replaced α-tocopherol in this action.

The ability of α-tocopherol or xanthine to improve casein utilization may be related to the report of Schwarz ('44) that these compounds prevented cirrhotic changes in the liver and death of rats on a diet containing 15% alkali-extracted casein. Traces of xanthine contained in crude casein would be removed by the alkali extraction and a xanthine-free diet would result. Giri and Rao ('46) reported that xanthine functions as an antioxidant in metal catalyzed oxidations and it may be in this same capacity that it acts similarly and non-specifically with α-tocopherol *in vivo*.

Schwarz found dried yeast, 0.5 gm daily, to be but slightly active, while our results showed a yeast protein concentrate to be highly active. The active agent in our yeast preparation may have been liberated or formed from precursors during the enzymatic digestion. Studies on this problem as well as the influence of other purine bodies and of folic acid are being pursued. In this regard Bosshardt et al. ('46b) working with mice noted an increased utilization of casein protein produced by a butanol extract of liver.

On diets with casein levels as low as 5 or 6%, tocopherol did not increase the protein-efficiency ratio over the 4-week

test period. Rats kept on such diets for periods up to 20 weeks, however, showed a dramatic growth and survival benefit from α-tocopherol (Hove, '46). This difference may indicate that the interrelation between protein and α-tocopherol follows separate and independent mechanisms under these 2 experimental conditions.

SUMMARY

1. d, α-tocopherol increases the utilization of casein protein for growth of young rats when the casein level in the diet is between 6 and 12%. There is no benefit due to tocopherol at casein levels over 12% or under 6% in the 4-week test period.

2. d, α-tocopherol reduces the incidence of stomach ulcers and the bleaching of maxillary incisors which occur in rats maintained on low-casein diets.

3. A yeast-protein concentrate or 0.1% xanthine in the diet is as effective as α-tocopherol in increasing the utilization of casein protein.

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THE EFFECT OF FAT LEVEL OF THE DIET ON GENERAL NUTRITION

IV. THE COMPARATIVE COMPOSITION OF RATS IN RELATION TO INTAKE OF FAT AND CALORIES [1]

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Earlier studies from this laboratory have shown beneficial effects on rats of the inclusion of liberal amounts of fat in the diet. Growth, resistance to undernutrition, physical capacity, and reproductive performance were all improved on synthetic diets containing 10 to 40% of fat, as compared with similar diets lacking fat (Deuel et al., '47; Scheer et al., '47a, b, c).

The present report includes data on the content of water, protein, fat, ash, and calcium in the bodies of rats selected from these experiments. The methods used were essentially those employed by Deuel et al. ('44), but the entire body of the animals was analyzed, rather than the eviscerated carcass. The intestine was removed, and the contents were

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The work was also undertaken in cooperation with the Committee on Food Research of the Quartermaster Food and Container Institute for the Armed Forces. The opinions and conclusions contained in this report are those of the authors. They are not to be construed as necessarily reflecting the views or indorsement of the War Department.

washed out with saline; the intestine was then passed through the meat grinder together with the remainder of the rat body.

The composition of the diets used and general experimental procedure have been published previously (Deuel et al., '47). Briefly, the diets were composed entirely of purified or synthetic materials, in such proportions that the intake of protein, minerals and vitamins would bear a constant ratio to caloric intake. The protein content of the diet ranged from 25% to 37.5%. The fat contents varied as follows: 60a, no fat, but ethyl laurate (1%) added as carrier for fat-soluble vitamins; 60b, no fat, but methyl linolate (1%); 60c, no fat, but methyl linolate (2.5%); 61, 5% fat (cottonseed oil); 62, 10% fat; 63, 20% fat; 64, 40% fat. The stock diet contained oats, wheat, alfalfa, yeast, salts, and cottonseed oil; the fat content was about 14%, and protein about 15%.

RESULTS

The results of the body analyses are summarized in table 1. The values for rats fed *ad libitum* (series I) agree fairly well with the figures of Deuel et al. ('44), but the variability is greater in our values. The protein, ash and calcium contents of our males appear somewhat higher, the carbohydrate somewhat lower than those reported by Deuel et al., and the sex differences reported by these authors are not as evident in our data. In the earlier tests a uniform fat level was fed in all tests and a large number of rats were available for comparison of the influence of sex on body composition. Apparently, however, the inclusion of the viscera in the analysis does not greatly alter the values obtained.

A period of very severe caloric restriction in young adult rats previously fed the stock diet *ad libitum* evidently results in a decrease in fat and carbohydrate, and an increase in water and ash (series II). The protein content increases slightly. The fat content appeared to be slightly higher on diets 63 and 64 providing liberal fat intake than on fat-free or low-fat diets. This is correlated with the fact that mortality was least in animals receiving diets high in fat. How-

TABLE 1

The body composition of male and female rats. Series I were fed ad libitum on diets containing several levels of fat for 18 weeks after weaning; Series II were fed ad libitum on stock diet for 19 weeks, followed by diets containing several levels of fat at 24 cal. per day for 8 weeks and then 12 cal. per day for 4 weeks; Series III received 12 cal. per day for 12 weeks after weaning while Series IV were also fed at a level of 12 cal. per day for 12 weeks after weaning but then received the diets ad libitum for 4–7 weeks (females) or 7–10 weeks (males).

CATEGORY	SERIES	DIET 60a		DIET 60b OR 60c		DIET 61		DIET 62		DIET 63		DIET 64		STOCK DIET	
		♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
Total number analyzed	I	8	5	8 [1]	5 [1]	7	5	9	5	8	5	8	5	7	5
	II			3	5	1	5	3	4	3	4	6	3	6	3
	III			4	4	4	4	4	4	3	3	3	4	3	5
	IV			4	4	3		4	1	5	4	5	4	2	5
Body weight, gm	I	235	179	256 [1]	176 [1]	311	202	326	215	377	223	343	208	310	202
	II			122	109	100	111	120	124	145	124	142	108	153	132
	III			55	55	62	50	58	55	62	58	66	57	56	54
	IV			183	153	221		230	154	226	168	213	172	191	179
Water, %	I	58.2	60.6	60.5 [1]	61.5 [1]	57.0	58.8	54.6	50.9	52.2	56.7	55.2	56.8	58.8	59.2
	II			69.4	69.0	69.9	68.9	68.8	69.8	67.8	66.1	67.4	67.3	69.6	69.4
	III			68.3	67.3	67.7	64.6	71.9	68.3	68.8	65.1	67.1	66.8	73.0	66.2
	IV			65.9	63.0	66.4		64.7	63.1	64.0	61.3	68.0	60.7	65.8	64.7
Protein, %	I	19.6	16.0	19.4 [1]	15.0 [1]	18.7	16.5	17.6	17.4	17.9	16.5	18.7	17.1	18.2	17.4
	II			20.9	19.9	20.2	19.6	21.6	19.5	21.0	20.5	20.9	20.2	20.5	19.8
	III			17.3	16.5	16.5	16.9	14.1	16.8	16.2	18.0	18.3	16.2	16.8	16.3
	IV			17.1	15.3	16.6		17.3	16.1	16.6	16.1	16.3	17.3	17.2	17.1
Fat, %	I	16.2	18.3	14.2 [1]	16.4 [1]	19.4	19.1	22.5	25.5	24.7	21.7	21.0	20.4	9.9	9.7
	II			1.2	2.4	1.0	2.1	1.3	3.1	3.2	5.1	3.5	5.3	1.8	2.5
	III			8.1	9.5	9.1	11.7	8.1	8.4	8.6	9.2	11.9	9.8	4.7	9.0
	IV			11.1	16.5	11.6		11.7	15.5	13.5	17.4	10.2	16.0	12.0	12.9
Carbohydrate, %	I	2.3	1.6	2.4 [1]	3.8 [1]	1.6	2.0	2.0	3.0	2.0	1.5	2.1	2.2	10.0	10.4
	II			3.5	3.8	2.3	4.1	2.6	3.3	3.4	2.5	3.0	3.0	2.9	3.3
	III			2.6	3.1	3.0	3.6	2.0	3.6	2.5	3.9	0.0	3.5	1.8	4.5
	IV			2.8	1.9	2.8		3.8	1.9	2.8	2.1	2.7	2.4	2.0	1.6
Ash, %	I	3.7	3.5	3.5 [1]	3.3 [1]	3.3	3.6	3.3	3.2	3.2	3.6	3.0	3.5	3.1	3.3
	II			5.0	4.9	6.6	5.3	5.7	5.2	4.6	5.0	5.2	4.2	5.2	5.0
	III			3.7	3.6	3.7	3.2	3.9	3.9	3.9	3.8	3.3	3.7	3.7	4.0
	IV			3.1	3.3	2.6		2.5	3.0	3.1	3.1	2.8	3.6	3.0	3.7
Calcium, %	I	1.52	1.60	1.44 [1]	1.66 [1]	1.39	1.70	1.22	1.34	1.38	1.58	1.15	1.50	1.30	1.44
	II			1.94	1.79	2.20	1.78	1.89	1.45	1.56	1.76	1.77	1.58	1.62	1.52
	III			1.23	1.15	1.18	1.02	1.16	1.20	0.90	1.22	1.17	1.09	1.15	1.37
	IV			0.93	1.09	0.72		0.64		0.93	0.88	0.78	1.05	0.95	1.10

[1] Received diet 60b.

ever, it should be emphasized that mortality was also low on the stock diet, and that the fat content of animals on this diet fell to low levels. In an earlier report (Scheer et al., '47c) we have suggested that the mortality observed was somehow related to vitamin deficiencies.

Rats kept on a restricted caloric intake for 12 weeks after weaning (series III) have a higher content of water, and less fat than do animals fed *ad libitum;* protein content is somewhat lower in the males, but not in females. There is no clear relation of fat content of the body to that of the diet. When these animals on restricted feeding were transferred to *ad libitum* feeding (series IV), there were small decreases in water and ash content, and definite increases in fat content on all diets. The fat content of the body does not appear to be correlated with the level of dietary fat. A slight decrease in carbohydrate is apparent in females, but not in males.

Taken together, our data show a very striking uniformity of body composition with gross variations in fat content of the diet and caloric intake. Fat and water content are the greatest variables, with protein remaining nearly the same throughout. In an earlier report (Deuel et al., '47) the greatest increase in body size in male rats was found on diets containing about 20% fat. This same diet results in the greatest deposition of body fat. However, the differences in weight on the various diets are not simply the result of differences in fat deposition. In fact, the relative contributions of the various components to the increased size, above that on a fat-free diet, are remarkably uniform. Table 2 presents data showing the difference in weight between rats receiving diets with fat and rats fed the fat-free ration (60b), and the percentage contribution to this increase of each of the body components analyzed for, this being determined by dividing the difference in total weight of each component by the difference in animal weight.

An earlier report (Scheer et al., '47b) showed that physical capacity is greater in rats fed liberal amounts of fat than in rats fed the fat-free diet. There is no evidence from the

present results that this difference is related to body composition.

TABLE 2

Relative contributions of various components of the body to the differences in weight between male rats fed diets containing fat and male rats fed a fat-free diet (60b).

DIET	WEIGHT DIFFERENCE	CONTRIBUTION TO WEIGHT DIFFERENCE FROM				
		Water	Protein	Fat	Ash	Carbohydrate
	gm	%	%	%	%	%
61	55	40	15	43	2	0
62	70	33	10	54	3	0
63	121	35	14	47	2	2
64	87	39	16	42	1	2

SUMMARY

1. Data are presented on the body composition of rats fed diets varying in fat content from 0 to 40%, at normal and severely restricted levels of caloric intake.

2. In general, the fat content of the body is not related to fat content of the diet; rats receiving diets with 20% fat fed *ad libitum* have the highest content of body fat.

3. Caloric restriction, in weanling or young adult rats, results in relatively high water content and very low fat content. Ash also increases in young adult rats.

4. The greater body size on diets containing liberal amounts of fat as compared with fat-free diets, is not the result solely of deposition of fat.

5. There is no evidence that the effects of varying fat content in the diet on resistance to undernutrition and on physical capacity are the result of differences in gross body composition.

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PEAS, SUPPLEMENTED WITH WHEAT GERM OR CORN GERM, AS A SOURCE OF PROTEIN FOR GROWTH[1]

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Legumes are readily obtained sources of vegetable protein and are frequently used in low cost diets. They are often used, also, to complete the protein quota in rations for both man and animals. The available supply of sulfur-containing amino acids limits the value of legumes as the sole source of protein. Woods, Beeson and Bolin ('43) and Lehrer, Woods and Beeson ('47) have shown that the growth efficiency of dry Alaska peas is more than doubled by the single addition of methionine to a 10% protein diet.

The cereal germs are another source of vegetable protein. As early as 1919 Osborne and Mendel reported that the proteins of the embryo of wheat were different chemically from those of the endosperm and were superior to those of the whole grain for maintenance of adult rats as well as for growth in the young. Stare and Hegsted ('44) reviewed the published reports on the protein of both wheat and corn germs and summarized their review by stating that these proteins were of "high nutritive value." Jones and Widness ('45, '46) obtained consistently better growth in weanling rats with wheat germ than with corn germ as the only source of protein.

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Modern milling methods produce both wheat and corn germs which are relatively free from other portions of the grain. These grain germs are available for both human and animal feeding.

The work reported here is a continuation of the study of the use of dry peas as a source of protein. This paper is concerned with the growth of rats produced by the combination of wheat or corn germ with peas.

EXPERIMENTAL PROCEDURE

Alaska field peas (23.1% protein) were soaked in distilled water overnight, cooked, dried at 62°C. and then ground. The wheat germ (28.8% protein), the usual commercial product, was ground in the laboratory, but not further processed. The corn germ [3] (23.2% protein) was a "low fat, dehydrated," finely ground product. A diet containing dried whole egg (44.0% protein) as the source of protein was used as a standard of comparison. All diets were made up to contain protein at a 10% level. The complete compositions of the diets are given in table 1.

Twenty-one gm of a vitamin-sucrose mixture and 2.5 gm choline chloride were included in each kg of diet. This amount of vitamin mixture provided the following supplements per 100 gm of diet: thiamine, 0.49 mg; riboflavin, 0.49 mg; pyridoxine, 0.61 mg; nicotinic acid, 0.61 mg; calcium pantothenate, 4.9 mg; para-amino benzoic acid, 29.4 mg; inositol, 98.0 mg; and 250 mg choline chloride. The Osborne and Mendel ('19) salt mixture was used in all diets. Methionine when used replaced an equal amount of sugar.

Weanling rats housed in individual cages were used for the investigation. They were weighed weekly for 8 weeks and food consumption records made at each weighing.[4]

The statistical analyses of the data were made according to the methods of Snedecor ('46).

[3] Generous amounts were furnished by the Vio Bin Corporation, Monticello, Illinois.

[4] The assistance of R. A. York in the care of animals is gratefully acknowledged.

TABLE 1

Composition of diets.[1] *The various sources of protein were used in amounts calculated to furnish protein at a 10% level.*

LOT NO.	PROTEIN		SUCROSE	SALT MIXTURE	COTTONSEED OIL	COD LIVER OIL
	Source	Amount				
		gm/kg	gm/kg	gm/kg	gm/kg	gm/kg
1	Egg[2]	227	639.5	40	30	20
2	Peas	433	444.5	40	39	20
3	Wheat germ	352	563.5	40	1	20
4	Corn germ	488	400.5	40	28	20
5	Peas	433				
	and		441.5	40	39	20
	dl-methionine	3				
6	50% Peas	195				
	and		512.5	40	14	20
	50% wheat germ	195				
7	75% Peas	310				
	and		481.5	40	22	20
	25% wheat germ	103				
8	87.5% Peas	372				
	and		465.5	40	26	20
	12.5% wheat germ	53				
9	50% Peas	231				
	and		425.5	40	29	20
	50% corn germ	231				
10	75% Peas	337.5				
	and		437.5	40	29	20
	25% corn germ	112.5				
11	87.5% Peas	389				
	and		442	40	30	20
	12.5% corn germ	55.5				

[1] Each diet contained 21 gm/kg of the vitamin-sucrose mixture described in the text in addition to 2.5 gm/kg of choline chloride.

[2] Egg diet contained 20 gm/kg of Agar-Agar.

RESULTS AND DISCUSSION

The results of these experiments are summarized in tables 2 and 3.

Examination of the results obtained from the diets containing a single product, as sole source of protein, reveals that the cereal germs compared favorably with the whole egg

TABLE 2

Average growth response.

LOT NO.	DIET	NO. OF RATS	AV. DAILY GAIN	AV. GAIN PER GM OF PROTEIN	AV. FOOD REQUIRED PER GM GAIN	AV. FOOD CONSUMED DAILY	AV. LIFE OF RATS [1]
			gm	*gm*	*gm*	*gm*	*days*
1	Egg	8	2.17 ± .11	2.50 ± .08	3.99 ± .20	8.65 ± .36	56
2	Peas	19	0.60 ± .04	0.95 ± .06	10.54 ± .68	6.36 ± .45	33
3	Wheat germ	12	2.37 ± .13	2.16 ± .09	4.73 ± .20	10.89 ± .12	56
4	Corn germ	12	2.36 ± .11	2.01 ± .06	5.01 ± .14	11.74 ± .13	56
5	Peas and 0.3% dl-methionine	19	1.88 ± .09	2.04 ± .06	4.89 ± .13	9.20 ± .39	56
6	50% Peas and 50% wheat germ	12	2.11 ± .11	1.96 ± .07	5.18 ± .20	10.74 ± .03	56
7	75% Peas and 25% wheat germ	7	1.77 ± .06	1.92 ± .05	5.23 ± .16	9.21 ± .02	56
8	87.5% Peas and 12.5% wheat germ	8	1.47 ± .04	1.69 ± .04	5.94 ± .15	8.75 ± .03	56
9	50% Peas and 50% corn germ	13	1.85 ± .11	1.97 ± .07	5.15 ± .19	9.33 ± .04	56
10	75% Peas and 25% corn germ	8	1.58 ± .05	1.75 ± .05	5.74 ± .14	9.02 ± .02	56
11	87.5% Peas and 12.5% corn germ	9	1.30 ± .08	1.51 ± .06	6.71 ± .24	8.64 ± .26	52

[1] Experimental period 56 days.

TABLE 3

Statistical comparisons of growth on various diets.

LOT NO.	DIETS COMPARED	AVERAGE DAILY GAIN (GM)			AVERAGE GAIN PER GM PROTEIN (GM)		
		Mean difference	Standard error of difference	t [1]	Mean difference	Standard error of difference	t [1]
1–2	Egg vs. peas	1.57	.12	13.08	1.55	.10	15.50
1–3	Egg vs. wheat germ	0.20	.16	1.22	0.34	.12	2.82
1–4	Egg vs. corn germ	0.19	.15	1.22	0.49	.10	4.90
1–5	Egg vs. peas + 0.3% dl-methionine	0.29	.14	2.07	0.46	.11	4.18
3–4	Wheat germ vs. corn germ	0.01	.17	0.05	0.15	.11	1.38
2–5	Peas vs. peas + 0.3% dl-methionine	1.28	.10	12.80	1.09	.09	12.11
5–6	Peas + 0.3% dl-methionine vs. 50% peas + 50% wheat germ	0.23	.14	1.62	0.08	.09	0.87
5–7	Peas + 0.3% dl-methionine vs. 75% peas + 25% wheat germ	0.11	.10	1.66	0.12	.08	1.53
5–8	Peas + 0.3% dl-methionine vs. 87.5% peas + 12.5% wheat germ	0.41	.09	4.16	0.35	.07	4.85
5–9	Peas + 0.3% dl-methionine vs. 50% peas + 50% corn germ	0.03	.14	0.02	0.07	.09	0.76
5–10	Peas + 0.3% dl-methionine vs. 75% peas + 25% corn germ	0.30	.10	2.91	0.29	.08	3.71
5–11	Peas + 0.3% dl-methionine vs. 87.5% peas + 12.5% corn germ	0.58	.12	4.81	0.53	.08	6.24
6–9	50% Peas + 50% wheat germ vs. 50% peas + 50% corn germ	0.26	.15	1.67	0.01	.09	1.01
7–10	75% Peas + 25% wheat germ vs. 75% peas + 25% corn germ	0.19	.07	2.43	0.17	.07	2.40
8–11	87.5% Peas + 12.5% wheat germ vs. 87.5% peas + 12.5% corn germ	0.17	.08	1.90	0.18	.07	2.49

[1] A t value of 3.499 establishes the 1% point for the comparisons based on the smallest number degrees of freedom; therefore a value of 3.499 or larger is highly significant.

in rate of gain but were less efficient. In the case of corn germ this difference is statistically significant. A comparison of the pea diet data with those of whole egg emphasizes the superior quality of egg protein. As previously reported by Jones and Widness ('46) the growth response resulting from wheat germ protein was better than that from corn germ. In the experiments reported here, the *t* value does not indicate that the difference is significant.

It has already been reported from this laboratory (Lehrer et al., '47) that the addition of 0.3% dl-methionine improves both growth rate and efficiency of food utilization on a diet in which cooked peas supply the protein. Results from the diets in which the protein is supplied by mixtures of peas and either wheat or corn germ also show improved growth-promoting quality. Both rate of gain and efficiency were not different from those of the methionine diet when peas and either germ were used in equal proportions, or when the mixture contained 25% wheat germ and 75% peas. However, when the mixture was 25% corn germ and 75% peas, the efficiency of the mixed proteins was significantly lower than that for the peas and methionine diet. When the mixture was reduced to 12.5% of either cereal germ to 87.5% of peas neither one gave as good growth as the 0.3% methionine supplemented pea diet. This is true whether the measure used was average daily gain or average gain per gm of protein.

SUMMARY AND CONCLUSIONS

A study of the quality of the proteins of wheat germ and of corn germ as a supplement for peas has been made using the growth-promoting method with young rats.

When used as the sole source of protein both of the products studied compared favorably with whole egg protein in rate of gain but the corn germ was significantly less efficient.

As a supplement for whole peas, wheat germ in equal proportions or as 25% wheat germ to 75% cooked peas had as good a growth-promoting value as peas supplemented with 0.3% dl-methionine.

Corn germ, fed in equal proportions with peas, produced as good results as the methionine diet but was not as efficient as methionine when used as 25% of the mixture.

Neither product as 12.5% to 87.5% peas gave as good results as 0.3% dl-methionine added to peas.

Under the conditions of this experiment, the wheat germ product used was a better supplement for peas than the corn germ.

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STUDIES ON THE CONCENTRATION OF SOME B-VITAMINS IN THE BLOOD OF NORMAL AND COBALT DEFICIENT SHEEP [1, 2]

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In a previous paper (Ray et al., '47), it was shown that injection of thiamine and possibly pyridoxine ameliorated the conditon of cobalt deficiency in ruminants. It was suggested that in cobalt deficiency, the production of B-vitamins in the rumen of the deficient animal was disturbed and the symptoms of the deficiency may be due to avitaminosis. In order to check this point, it was decided to examine the concentration of some B-vitamins in the blood of normal and cobalt deficient sheep, as it was thought that if these vitamins were not being elaborated in sufficient quantity in the rumen of the deficient animal, their concentration in the blood would also fall. The results of this investigation are reported here.

EXPERIMENTAL

The cobalt deficient sheep were all kept on a basal ration of cobalt deficient hay (Co content = 0.03 ppm) and a mixture of 80% corn and 20% corn gluten meal. In addition, the animals were offered iodized salt ad libitum. Some of these animals were born at the Experiment Station and had been

[1] Published with the approval of the Director of Wisconsin Agricultural Experiment Station.

[2] This work was supported in part by the Wisconsin Alumni Research Foundation.

on the cobalt deficient ration since their birth. The other animals of this group were purchased from farms where symptoms of cobalt deficiency had been prevalent and no cobalt therapy had been used. The normal animals were also kept on the identical ration except that they received cobaltized salt (12.7 gm $CoSO_4 \cdot 7H_2O$ per 25 lbs. iodized salt). The composition of the basal ration was kept constant in both groups, as it had been found by Lardinois et al. ('44), that the composition of the feed has a great effect on the production of B-vitamins in the rumen. The animals were kept on these rations for 6 months to 1 year before they were examined. In the deficient group, blood was collected from only those animals which were just beginning to show deficiency symptoms, as evidenced by a slowing of the growth rate. At this stage, the food intake of the deficient animals was quite comparable to that of the normal animals. This policy was adopted as we did not want to have the results complicated by inanition, which is always seen in acute cobalt deficiency.

Oxalated blood was collected in the morning and was sent under ice to the laboratory where it was kept in the refrigerator until used. In no case was the blood more than 3 days old when examined. One ml of blood was mixed with 7 ml of acetate buffer (pH 4.5), 1 ml of takadiastase solution (20 mg per ml), and 1 ml of papain suspended in water (20 mg per ml) (Cheldelin et al., '42). A little benzene was added as a preservative to the mixture which was then incubated for 24 hours at 37°C. The mixture was then heated for one-half hour in flowing steam, cooled, and filtered. An attempt was made to estimate the vitamins in this filtrate by the recognized microbiological methods, but too low recovery and too much "drifting" was observed. A known volume of filtrate was therefore extracted with twice its volume of ether and the ethereal layer was discarded. The extraction was carried out once more and the aqueous layer was freed of ether by gentle heating on a hot plate and then made up to proper volume. This procedure eliminated entirely the "drifting" seen with

increased amount of unknown solutions. The recovery of added vitamins was also good, being mostly within 92–103%. Roberts and Snell's method ('46), using *L. casei* was used for the determination of riboflavin, pantothenic acid, folic acid, and niacin. For the estimation of total pyridoxine, the method of Atkin et al. ('43), as modified by Rabinowitz and Snell ('47), was employed, using *Saccharomyces Carlsbergensis* as the microorganism. For this last method, 1 ml of blood was mixed with 9 ml of 0.05 N HCl, autoclaved for 5 hours at 15 lbs. pressure, cooled, adjusted to pH 5.2, centrifuged, and the clear supernatant fluid used for analysis. With both *L. casei* and the yeast, an 18-hour incubation, followed by the turbidimetric measurement of the growth of microorganisms was used. In pyridoxine determination, the tubes were shaken continuously during the whole period of incubation.

The results of our findings are given in table 1.

TABLE 1

Concentration of various vitamins in the blood of normal and cobalt-deficient sheep. All values are in μg per ml of blood, with means and standard deviations from the mean.

VITAMIN	NORMAL	DEFICIENT
Riboflavin	0.265 ± 0.028 (13)[1]	0.235 ± 0.035 (12)
Folic acid	0.046 ± 0.008 (8)	0.044 ± 0.007 (6)
Pantothenic acid	0.418 ± 0.070 (10)	0.432 ± 0.082 (13)
Nicotinic acid	12.14 ± 1.81 (8)	9.53 ± 2.99 (6)
Vitamin B_6 group	0.118 ± 0.018 (6)	0.067 ± 0.010 (5)

[1] The figures in parentheses represent number of animals examined.

DISCUSSION

No values for the riboflavin, folic acid, and vitamin B_6 contents for normal sheep blood are available in the literature. Our values for the riboflavin content for normal sheep are lower than those reported in normal animals of other species by Strong et al. ('41); but as these authors did not carry out any lipid separation from the blood extract, the values found by them might have been too high (Eckhardt et al., '41). Our

folic acid values are similar to those found for man, but higher than those of cattle or pigs (Schweigert and Pearson, '47). Our pantothenic acid values are much higher than those reported by Pearson ('41) for sheep. This may possibly be due to a difference in the rations of Pearson's animals, as it has been shown by Lardinois et al. ('46), that ration composition influences greatly the rumen synthesis of the vitamin-B complex. Our values for the nicotinic acid content of normal sheep blood are similar to those found by Pearson et al. ('40).

It may be seen that the concentrations of nicotinic acid and vitamin B_6 contents of the blood of deficient animals were definitely lower than those in normal sheep; there was a suggestion of a similar lowering in the case of riboflavin. It is plausible, therefore, that these 3 vitamins were not being produced in sufficient quantity in the rumen of the cobalt deficient sheep. The folic acid and pantothenic acid contents were, however, nearly the same in the 2 groups. This may indicate that the production of various members of vitamin-B complex may be brought about by different rumen microflora and cobalt may influence the production of some, but not of all the members.

SUMMARY

The concentrations of nicotinic acid and vitamin B_6 group in the blood of cobalt deficient sheep were found to be lower than those in the normal animals; there was a suggestion of a similar lowering in the case of riboflavin. The folic acid and pantothenic acid contents were not affected.

The relation of cobalt metabolism in ruminants to endogenous production of certain of the B-vitamins is discussed.

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MEAD JOHNSON AND COMPANY 'B-COMPLEX' AWARD

Nominations are solicited for the 1948 Award of $1,000 established by Mead Johnson and Company to promote researches dealing with the B complex vitamins. The recipient of this Award will be chosen by a Committee of Judges of the American Institute of Nutrition and the formal presentation will be made at the annual meeting of the Institute in the Spring of 1948.

The Award will be given to the laboratory (non-clinical) or clinical research worker in the United States or Canada who, in the opinion of the judges, has published during the previous calendar year January 1 to December 31 the most meritorious scientific report dealing with the field of the 'B-complex' vitamins. While the award will be given primarily for publication of specific papers, the judges are given considerable latitude in the exercise of their function. If in their judgment circumstances and justice so dictate, it may be recommended that the award be made to a worker for valuable contributions over an extended period but not necessarily representative of a given year. Membership in the American Institute of Nutrition is not a requisite of eligibility for the award.

To be considered by the Committee of Judges, nominations for this award for work published in 1947 must be in the hands of the Nominating Committee by January 10, 1948. The nominations should be accompanied by such data relative to the nominee and his research as will facilitate the task of the Committee of Judges in its consideration of the nomination.

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